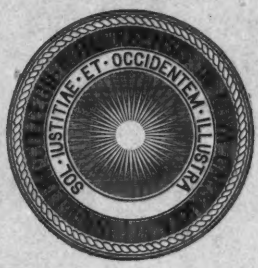
VOL. VI JULY, 1918 No. 1

SOIL SCIENCE

FOUNDED BY

RUTGERS COLLEGE

NEW BRUNSWICK, N. J.



JACOB G. LIPMAN, Editor-in-Chief

CARL R. WOODWARD, Assistant Editor

PUBLISHED MONTHLY BY
WILLIAMS & WILKINS COMPANY
BALTIMORE, MD., U. S. A.

THE CAMBRIDGE UNIVERSITY PRESS
FETTER LANE, LONDON, E. C.

Entered as second-class matter August 23, 1917, at the post office at Baltimore, Maryland, under the act of March 3, 1879.

Price { $5.00 per year, two volumes, United States
$5.25 per year, two volumes, Canada
$5.50 per year, two volumes, other countries

SOIL SCIENCE

Contents for July, 1918

A COMPARATIVE STUDY OF SALT REQUIREMENTS FOR YOUNG AND FOR MATURE BUCKWHEAT PLANTS IN SAND CULTURES

JOHN W. SHIVE

Laboratory of Plant Physiology, New Jersey Agricultural Experiment Station

Received for publication June 10, 1918

In a recent paper (9) presenting the results of a comparative study of salt requirements for buckwheat plants grown in solution cultures during two different periods of their development, it was announced that similar experiments were carried out with these plants in sand cultures corresponding to the solution cultures. The results of this experimental work with buckwheat in sand cultures furnishes the subject matter of the present paper, which also compares these results with those obtained from the corresponding solution cultures of the earlier work.[1]

As previously stated, the object of these investigations was to determine the salt requirements demanded for approximately optimum growth of buckwheat plants during the early stages of their development while the vegetative processes are extremely active, and to compare these with the relative salt proportions best adapted to the development of these plants during the reproductive stages and during the period of seed formation. The entire active life period of the plants was thus divided into two partial periods extending over nearly equal intervals of time, these partial periods representing distinct physiological phases of development. The first of these covered the period between the germination of the seeds and the beginning of the flowering stage, while the second extended from the close of the first period to the maturity of the seeds.

EXPERIMENTAL PROCEDURE AND METHODS

1. The culture solutions

Throughout these tests an optimal series of 3-salt solutions (7) was employed in sand cultures. This series was composed of 36 different solutions including all the possible proportions of the three salts, mono-potassium phosphate, calcium nitrate, and magnesium sulfate, when the partial concentrations of each of the components were made to vary by equal increments of one-tenth

[1] A preliminary report giving some of the principal results of these studies has already appeared: Shive, J. W., and Martin, W. M., Ref. No. 8.

of the total osmotic concentration. Each solution had an initial total concentration value of approximately 1.75 atmospheres. Previous experiments have shown that this concentration is well within the range required for optimum growth of buckwheat. The partial osmotic concentration values, as well as the volume-molecular partial concentrations of each salt in these solutions, have been calculated according to the method employed by Tottingham (10, p. 177–182 and 192). A table giving the formulas of the solutions of this series has already appeared in several publications (5, 7) and need not be repeated here. The methods used in preparing the stock solutions, and the necessary manipulations with reference to the making up of the nutrient solutions were practically the same as were those previously described (7).

2. The sand cultures with renewal of solutions

The substratum employed with the cultures of these tests consisted of white quartz sand which had previously been thoroughly washed with tap water followed with distilled water. For the first washing the sand was placed in a granite-ware tub and a stream of water from a hose was then directed into the sand, allowing the water to overflow the sides of the tub while the sand was constantly being agitated. This process was continued until the water overflowing the tub was clear of all sediment. The surplus tap water was then decanted, after which the sand was washed twice with distilled water by pouring the water on the sand in the tub and stirring the sand in this water. After the final washing with distilled water the sand was spread on large sheets of paper until air-dry. This sand had a water-holding capacity of 24.9 per cent (average of six determinations) on the dry-weight basis, determined according to the method of Hilgard (2), which employs sheet metal pans with lateral walls 1 cm. high, the bottoms being perforated. The percentages of different-sized particles of sand, obtained by a mechanical separation, are given in table 1.

TABLE 1

Mechanical analysis of sand giving the percentages of the different-sized particles

	per cent
Gravel (more than 2.0 mm.)	0.58
Fine gravel (2.0 mm. to 1.0 mm.)	5.67
Coarse sand (1.0 mm. to 0.5 mm.)	30.83
Medium sand (0.5 mm. to 0.317 mm.)	53.89
Fine sand (0.317 mm. to 0.254 mm.)	7.36
Very fine sand (less than 0.254 mm.)	1.67

The culture vessels consisted of glazed earthenware pots, each with a capacity of about 2 liters; 2.5 kgm. of sand were used for each culture. To provide for the removal of the solutions from the sand cultures, a method similar to that devised by McCall (4) was here adopted. A glass tube with an inside diameter of 4 mm. was placed vertically against the wall of the pot, extending through the sand to the bottom of the pot. The tube was screened at its

lower end by means of a plug of glass wool which prevented the escape of any grains of sand when suction was applied to the top of the tube. To prevent the plug from being drawn through the tube when suction was applied for the removal of the solution, a tuft of the glass wool attached to the plug was allowed to extend 1.5 cm. to 2.0 cm. beyond the end of the glass tube. The sand resting upon this tuft held the plug firmly in place. After the sand (2500 gm.) had been weighed into the pot, a paraffined paper funnel was placed in the inverted position at the center of the sand surface. The lower end of the funnel was buried in the sand to the depth of about 1 cm. These funnels, which were about 6 cm. in length with a diameter of 2 cm. at one end and about 4 cm. at the other, were made of heavy paper and were then thoroughly impregnated with melted paraffine to render them impervious to moisture. To prepare the dry sand in each pot for the planting of the seedlings, the nutrient solution was poured into the sand through the funnel until the sand was nearly saturated. The sand culture was then ready to receive the seedlings.

The "Japanese" variety of buckwheat was employed. The seed used was from the same lot as was used in the earlier work with solution cultures corresponding to the sand cultures of the present study. The seeds were germinated and the seedlings grown on a germinating net in the manner described in a previous publication (7). When the seedlings were about 5 cm. tall, those selected for uniformity were carefully transferred to the pots of sand which had previously been prepared in such a way as to provide for the renewal of the solutions in the sand at regular intervals during the growth period. Five seedlings were transplanted to each pot, after which a sufficient amount of the solution was added through the funnel to flood the culture until the free solution stood above the sand to the depth of 1 cm. or more. This served to fix the roots of the seedlings in place and to smooth the surface of the sand. With this initial application, 750 cc. of solution were added to the sand of each culture. Suction was then applied to the top of the glass tube (provided for the removal of the solution) by means of an aspirating pump and the withdrawal of the solution was continued until the sand was reduced to the desired moisture content, which throughout these tests was maintained at approximately 15 per cent of the dry weight of the sand, or 60 per cent of its water-holding capacity. Two more portions of the solution (250 cc. to each portion) were then passed through the sand, the moisture content being reduced each time to the desired 60 per cent of its moisture-holding capacity. The culture was then sealed. This was done by pouring over the surface of the sand a thin layer of melted Briggs and Shantz (1) wax, thus completely covering the sand between the walls of the pot and the funnel. The sealing of the cultures was rendered necessary in order to prevent evaporation and the consequent disturbing factor of salt precipitation at the surface of the sand, and in order also to control the concentration of the nutrient solution and to make possible the measurement of water loss by transpiration.

At the end of each three or four day period, each pot was weighed and a sufficient amount of distilled water was added through the funnel to restore the entire system to its original weight, after which a fresh nutrient solution was added (250 cc. to each culture) while at the same time an equal quantity was withdrawn through the glass tube provided for the purpose.

The transpirational water loss from the plants, during a given interval of time, is supplied, of course, through the absorption by the roots of an approximately equal quantity from the solution in the medium in which the plants are rooted. This process results in a gradual increase in the concentration of the solution. In order to prevent any excessive variations in the concentration of the nutrient solutions, due to the absorption by the roots, the cultures were weighed at 2-day intervals during the early growth stages and daily during the later stages of growth. At each weighing a sufficient quantity of distilled water was added to each culture to restore that which had been absorbed by the roots during the interval in question. The amount of water added to each culture at any weighing was approximately equal, of course, to the amount lost by transpiration during the interval directly preceding the weighing. A record was kept of the amounts of water added from time to time. The total amount of water lost during the entire growth period was obtained by summing the losses for the partial periods between each two successive weighings.

3. Early period of development

In order to determine the best proportions of the three salts KH_2PO_4, $Ca(NO_3)_2$, and $MgSO_4$, for the growth of buckwheat tops and of roots in sand cultures during the early developmental period, between the germination of the seed and the flowering stage, 36 sand cultures were prepared, as above described, with the 36 different solutions of the 3-salt series employed in these tests. All the seedlings used were selected for uniformity of size and vigor. These were carefully removed from the germination net, one at a time, when about 4 cm. tall, and were transplanted to the sand cultures previously prepared, 5 seedlings to each culture. One culture was prepared also with Knop's solution and another with Tottingham's best solution for wheat tops, each with a total osmotic concentration value (1.75 atmospheres) equal to that of the solutions of the 3-salt series. These cultures were added to the series for comparison.

This series of sand cultures was now continued with renewal of solutions every three or four days, until the plants began to bloom. This required a time period of 25 days after the seedlings had been transferred to the sand cultures. The series was then repeated.

At the end of the growth period the wax seal was removed from the cultures and the tops of the plants were severed from the roots just at the surface of the sand. The tops were then dried to constant weight at a temperature of

about 103°C. and the dry weights obtained. The method employed in harvesting the roots was essentially the same as that adopted by McCall (5). In order to wash the roots as free from sand as possible, the contents of each culture pot, after the tops of the plants had been removed, were transferred to a sieve with meshes sufficiently large to allow all the sand grains to pass through. The sand was then washed through the sieve by means of a gentle stream of water, leaving the roots on the sieve. The roots, together with some adhering sand grains, were dried to constant weight in the same manner as were the tops. The dried roots with the adhering sand were then weighed, after which the roots were ignited in crucibles of fused silica until all the organic matter had been destroyed. The loss in weight due to the ignition process was taken to represent the approximate dry weight of the roots, assuming, of course, that the adhering sand suffered no loss in weight in the ignition process. The small amount of ash resulting from the ignition of the roots was considered negligible, since, as McCall pointed out, the relative weights would be affected only by the differences between the weights of ash from the various individual cultures.

Throughout these experiments daily records were kept of the temperature and moisture conditions in the greenhouse where the cultures were conducted. Maximum and minimum temperature readings were obtained from thermometers protected from direct sunlight, and the evaporating power of the air was measured by means of the daily rates of water loss from standardized, spherical, porous-cup atmometers. The readings obtained from these instruments were corrected to the Livingston (3) standard spherical cup by multiplying the actual readings by the coefficient of correction of the cups used.

The first of the two series conducted during the early developmental period extended from April 12 to May 7, 1917. During the period of this series the highest temperature recorded was 33°C. (on April 23), and the lowest was 7°C. (on April 18). The water loss from the porous cup atmometer gave a daily mean of 9.8 cc., a maximum daily rate of 20.9 cc. (on April 18), a minimum daily rate of 2.9 cc. (on April 27), and a total loss from the instrument of 244.6 cc. for the entire period. The second series, which was just like the first, was carried out between May 24 and June 18, 1917. During this time the maximum temperature reached was 35°C. (on June 11), and the minimum was 9°C. (on May 26). The evaporation rate from the atmometer gave a daily mean of 12.6 cc., a maximum daily rate of 21.6 cc. (on June 4), a minimum daily rate of 2.0 cc. (on May 29), and a total loss of 313.6 cc. for the entire period. For the sake of convenience in presenting the data, this double series will be designated series A throughout.

4. Late period of development

The tests dealing with the questions of salt requirements for the buckwheat during the later growth period were begun with plants which had reached the stage of development attained by those harvested at the end of the early

period of growth, when the plants had just reached the flowering stage. In order to obtain a sufficient number of plants at this stage of development, which were all nearly alike in size and vigor, the following procedure was adopted:

A larger number of sand cultures than that required for the series was prepared. Carefully selected seedlings were transplanted to these cultures, five seedlings to each culture, in the manner above described. Each of these sand cultures was provided with the solution[2] of the optimal 3-salt series producing the highest yield of buckwheat tops and roots during the first four weeks after germination. These cultures were continued to the flowering stage, with renewal of solutions every third or fourth day, covering an early growth period of 25 days after the seedlings had been transferred to the sand cultures. During this time all the plants were grown in the same nutritive medium and under approximately similar conditions of temperature, light, and moisture. This procedure gave very uniform plants.

At the end of the early 25-day growth period, 36 cultures were selected from the larger number at hand. The solutions in these and cultures were now replaced by the 36 different solutions of the optimal 3-salt series. This was accomplished by passing through the sand of each culture (after first adding sufficient distilled water to bring the entire system back to its original weight) a triple portion (750 cc.) of the new solution, thus flushing out the old solution and replacing it with the new. One culture with Knop's solution and one with Tottingham's best solution for wheat tops were also included in the series for comparison. These were treated in the same manner as were the other cultures of the series. The series was now continued with renewal of solutions every third or fourth day as before, until practically all the seeds were ripe. This second, or late, developmental period extended over a time interval of 30 days. The entire active growth period of the plants, after the seedlings had been transferred to the sand cultures, extended over an interval of 55 days.

At the end of the active growth period the plants were harvested and the dry weight yields obtained in the same manner as were those of series A, representing the early developmental period. The yields of tops, roots and seeds were obtained separately.

During the active growth period, extending from April 25 to June 19, the maximum temperature experienced by the cultures was 35°C. (on June 11), and the minimum was 9°C. (on May 26). The water loss from the atmometer, indicating the evaporating power of the air, gave a daily mean of 12.4 cc., a maximum and a minimum daily rate of 23.2 cc. and 2.0 cc. on June 5 and May 29, respectively, and a total loss from the instrument of 680.2 cc. for the entire time period. The series was repeated between July 2 and August 27. During this time a maximum temperature of 38°C.

[2] This solution contained the three salts in the following volume-molecular partial concentrations: KH_2PO_4, 0.144 m.; $Ca(NO_3)_2$, 0.0052 m.; and $MgSO_4$, 0.0200 m.

was reached on August 1, and a minimum of 14° C. on July 12. The rate of evaporation from the atmometer gave a daily mean of 17.1 cc., a maximum and a minimum daily rate of 35.1 cc. and 3.3 cc. on August 26 and July 12, respectively, and a total loss of 938.9 cc. for the entire time period. This double series will be designated series B, throughout this study.

EXPERIMENTAL RESULTS

As previously stated, a comparative study of the growth of buckwheat plants in water cultures, corresponding to the present study with sand cultures, has already been carried out. The methods of presenting the results obtained with the two double series of cultures here considered will, therefore, follow the same general outline as that employed in the earlier work (9).

The behavior of the *young* buckwheat plants (series A, early developmental period) in the different sand cultures provided with the solutions of the optimal 3-salt series, will be compared with the behavior of the *older* plants (series B, late period of development) grown in sand cultures provided with solutions of the same series. The comparison will be made by means of three kinds of direct quantitative measurements: (1) dry weights of tops, (2) dry weights of roots, and (3) total transpirational water loss from the plants during the entire growth periods. Further comparisons will be made of two other quantitative criteria derived from the transpiration values considered in connection with the dry weights of tops and of roots. These are (1) water requirements of tops and (2) water requirements of roots. These values represent the transpirational water loss for each single gram of dry plant substance produced. The dry weights of seeds will also be considered in connection with the data of series B.

I. Dry-weight yields

A. Presentation of data

Since the tops and roots of series A, and the tops, roots, and seeds of series B were weighed separately, two sets of dry-weight measurements are available for the former series and three for the latter. The dry-weight values of tops and of roots of series A are presented in table 2, and in a similar manner those of series B are given in table 3. In the first column of each table are given the culture numbers referring to the positions which the cultures occupy on the triangular diagram[3] graphically representing the variations in the salt proportions of the culture solutions of the series here employed in sand cultures.

Since, as above stated, each of the two series was repeated, each dry weight datum, as given in the tables, represents the average yield obtained from two corresponding cultures. The tables give the average absolute dry weights, in grams, and also the relative values of these in terms of the corresponding value

[3] For the description of this diagram see Shive (7) and McCall (5).

TABLE 2

Average dry weights of tops and roots of buckwheat grown to the flowering stage in sand cultures supplied with three-salt solutions, all having a total osmotic concentration value of 1.75 atmospheres, but differing from each other in the proportions of the constituent salts

CULTURE NUMBER	AVERAGE DRY-WEIGHT YIELDS			
	Tops (5 plants)		Roots (5 plants)	
	Absolute	Relative to R_1C_1 as unity	Absolute	Relative to R_1C_1 as unity.
	gm.		*gm.*	
R_1C_1	1.875	1.00	0.276	1.00
C_2	2.850	1.52	0.292	1.06
C_3	2.858	1.52	0.250	0.91
C_4	3.145	1.68	0.334	1.21
C_5	3.072	1.64	0.328	1.19
C_6	3.186	1.70	0.333	1.21
C_7	3.642	1.94	0.363	1.31
C_8	2.860	1.52	0.294	1.06
R_2C_1	2.023	1.08	0.224	0.81
C_2	3.492	1.86	0.382	1.38
C_3	3.648	1.95	0.376	1.36
C_4	3.423	1.82	0.316	1.15
C_5	3.566	1.90	0.384	1.39
C_6	3.854	2.05	0.409	1.48
C_7	2.803	1.49	0.318	1.15
R_3C_1	2.560	1.36	0.279	1.01
C_2	3.825	2.04	0.312	1.13
C_3	3.542	1.89	0.427	1.55
C_4	3.456	1.84	0.406	1.47
C_5	3.508	1.87	0.391	1.42
C_6	3.164	1.69	0.317	1.15
R_4C_1	2.695	1.44	0.339	1.23
C_2	4.457	**2.38**	0.373	1.35
C_3	4.141	2.20	0.388	1.41
C_4	3.748	2.00	0.402	1.46
C_5	3.746	2.00	0.398	1.44
R_5C_1	2.228	1.19	0.235	0.85
C_2	2.971	1.58	0.287	1.04
C_3	3.888	2.08	0.319	1.15
C_4	3.707	1.98	0.456	**1.65**
R_6C_1	2.382	1.27	0.298	1.08
C_2	3.397	1.81	0.399	1.44
C_3	3.472	1.85	0.358	1.30
R_7C_1	2.419	1.29	0.291	1.05
C_2	2.996	1.60	0.316	1.14
R_8C_1	2.515	1.34	0.200	0.73
K*	3.187	1.70	0.319	1.15
T*	3.577	1.91	0.392	1.42

* In this table and in subsequent tables K and T represent cultures prepared with Knop's solution and with Tottingham's best solution, respectively. The data obtained from these cultures are introduced for comparison.

TABLE 3

Average dry weights of tops, roots, and seeds of buckwheat grown from the flowering stage to maturity in sand cultures supplied with three-salt solutions, all having a total osmotic concentration value of 1.75 atmospheres but differing from each other in the proportions of the constituent salts

CULTURE NUMBER	AVERAGE DRY-WEIGHT YIELDS						RATIO OF TOPS TO SEEDS
	Tops (5 plants)		Roots (5 plants)		Seeds (5 plants)		
	Absolute	Relative to R_1C_1 as unity	Absolute	Relative to R_1C_1 as unity	Absolute	Relative to R_1C_1 as unity	
	gm.		*gm.*		*gm.*		
R_1C_1	5.345	1.00	0.690	1.00	2.980	1.00	1.79
C_2	6.346	1.17	0.783	1.13	3.332	1.12	1.91
C_3	7.696	1.44	1.003	1.45	4.328	1.45	1.78
C_4	8.825	1.65	0.905	1.31	4.694	1.57	1.88
C_5	8.957	1.68	0.935	1.36	4.306	1.45	2.08
C_6	7.523	1.40	0.723	1.05	3.696	1.24	2.03
C_7	8.168	1.53	0.828	1.20	2.804	0.94	2.92
C_8	9.287	1.72	0.812	1.18	2.681	0.90	3.46
R_2C_1	5.599	1.05	0.680	0.99	2.246	0.76	2.49
C_2	6.678	1.25	0.737	1.07	2.137	0.72	3.12
C_3	8.863	1.66	0.985	1.43	3.176	1.07	2.78
C_4	8.889	1.66	1.029	1.49	3.895	1.31	2.28
C_5	9.364	1.75	1.142	1.65	4.230	1.42	2.21
C_6	9.599	1.80	1.132	1.64	3.058	1.03	3.14
C_7	8.135	1.52	0.926	1.34	3.260	1.09	2.51
R_3C_1	7.911	1.48	0.691	1.00	2.120	0.71	3.73
C_2	8.492	1.59	0.651	0.94	2.647	0.89	3.20
C_3	8.544	1.60	1.157	1.68	3.174	1.06	2.69
C_4	10.298	1.93	1.114	1.63	4.249	1.43	2.42
C_5	12.314	**2.30**	1.300	**1.88**	5.001	**1.68**	2.46
C_6	9.501	1.78	1.117	1.62	4.304	1.44	2.21
R_4C_1	6.783	1.27	0.768	1.11	2.325	0.78	2.92
C_2	8.316	1.56	1.063	1.54	3.752	1.26	2.22
C_3	8.726	1.64	0.886	1.28	4.190	1.41	2.08
C_4	9.853	1.84	1.215	1.76	3.238	1.09	3.04
C_5	6.831	1.28	0.805	1.17	3.122	1.05	2.19
R_5C_1	5.528	1.03	0.795	1.15	3.651	1.22	1.51
C_2	8.772	1.64	1.017	1.47	2.204	0.74	3.98
C_3	8.943	1.67	1.087	1.58	3.594	1.20	2.49
C_4	9.160	1.71	0.873	1.27	2.657	0.89	3.44
R_6C_1	6.000	1.12	0.788	1.14	2.754	0.92	2.18
C_2	8.255	1.55	1.085	1.57	3.761	1.26	2.20
C_3	7.973	1.49	0.535	0.78	3.257	1.09	2.44
R_7C_1	6.231	1.16	0.802	1.16	3.105	1.04	2.00
C_2	7.023	1.31	0.682	0.97	3.250	1.09	2.16
R_8C_1	5.897	1.10	0.677	0.98	2.830	0.95	2.08
K	8.708	1.63	1.070	1.55	4.046	1.36	2.15
T	8.737	1.64	0.907	1.31	3.456	1.16	2.53

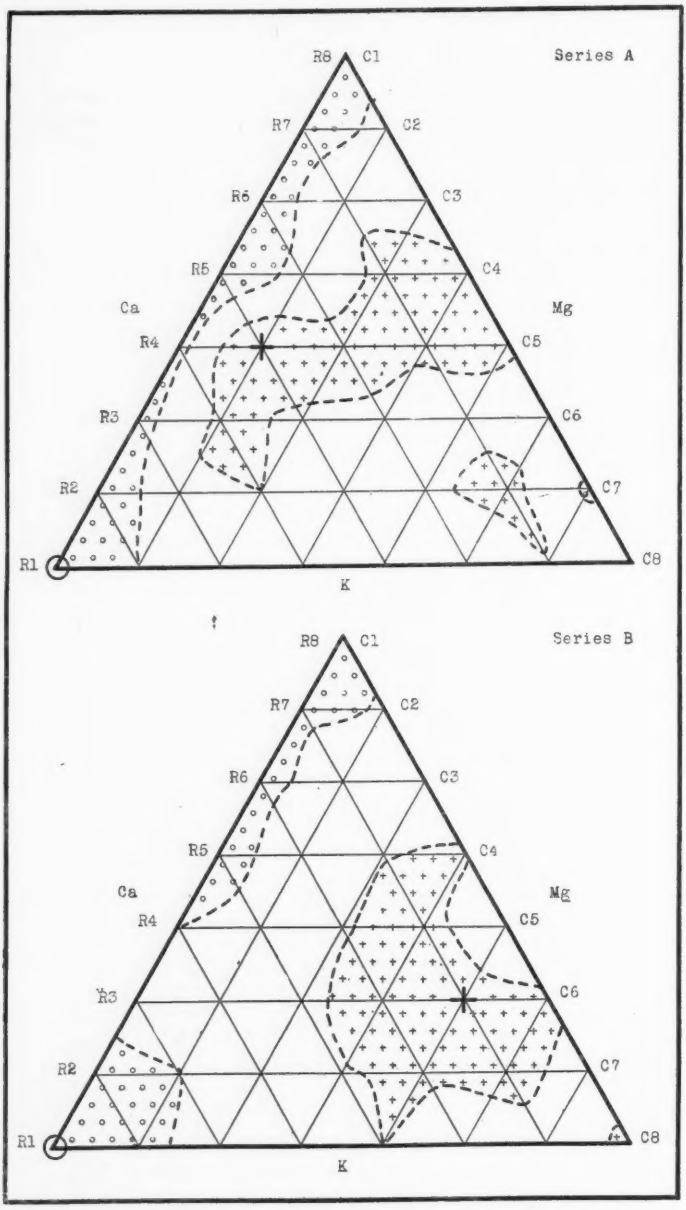


FIG. 1. DIAGRAMS SHOWING RELATIVE YIELDS OF BUCKWHEAT TOPS

Areas of high yields indicated by small crosses, those of low yields by small circles. The culture of each diagram giving the highest yield is marked by a larger cross, that giving the lowest yield by a larger circle.

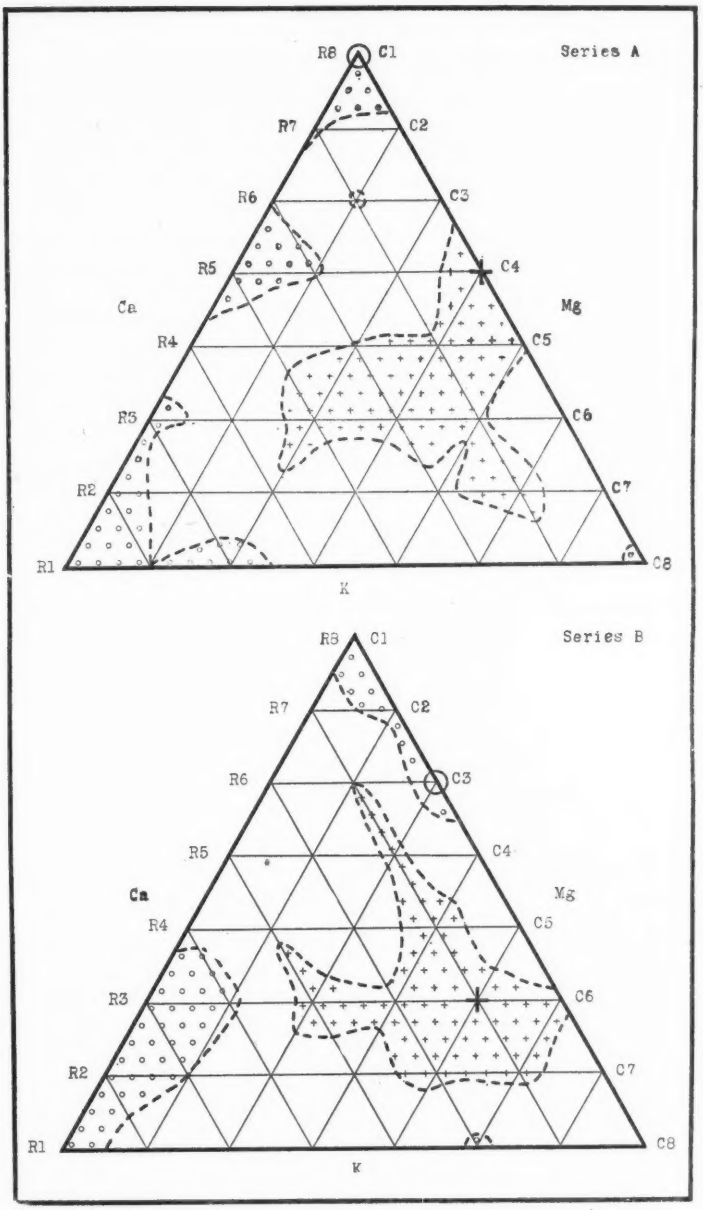


FIG. 2. DIAGRAMS SHOWING RELATIVE YIELDS OF BUCKWHEAT ROOTS

Areas of high yields indicated by small crosses, those of low yields by small circles. The culture of each diagram giving the highest yield is marked by a larger cross, that giving the lowest yield by a larger circle.

for culture R_1C_1 considered as 1.00. The highest relative yields are here indicated in black-face type. In the last column of table 3 are given the ratios of tops to seeds. The last two items in each column refer to the yields obtained from the sand cultures treated with Knop's solution and Tottingham's best solution for wheat, respectively. These were included in each series for comparison.

For facility in making comparisons, the relative yield values of series A and series B were here plotted on the triangular diagrams in the same manner as

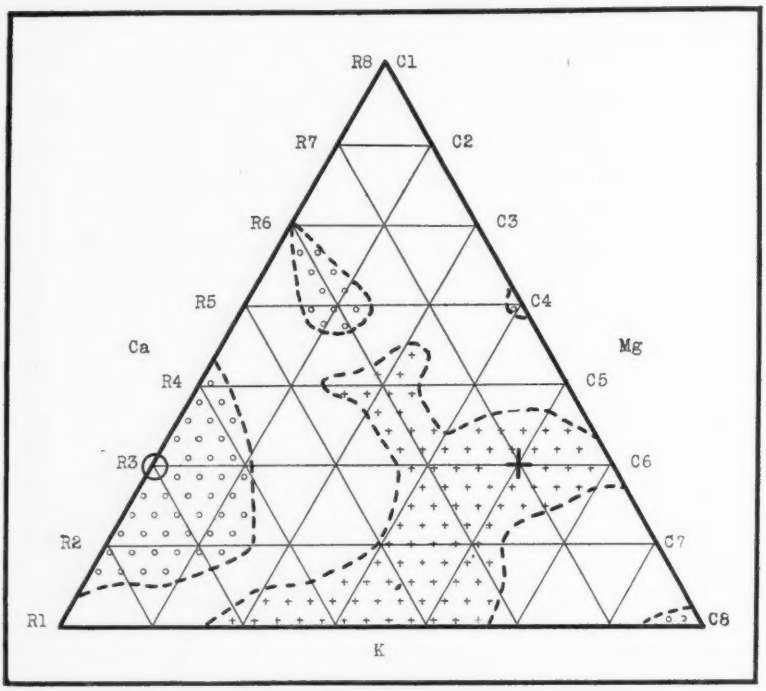


FIG. 3. DIAGRAM SHOWING RELATIVE YIELDS OF BUCKWHEAT SEEDS

Area of high yields indicated by small crosses; areas of low yields indicated by small circles. The culture giving the highest yield is marked by a larger cross, that giving the lowest yield by a larger circle.

were the yield values obtained from buckwheat plants grown in the culture solutions of the earlier work, corresponding to the sand cultures of the present study. At the intersections of the lines showing the culture locations on the diagrams of figures 1 to 3, were placed the numbers representing the average relative dry weights taken directly from the proper columns of tables 2 and 3.

Each diagram thus graphically represents the distribution of the yield values in its respective series. In order better to study the growth rates, each series of 36 cultures was divided into three groups. One group comprises the nine cultures giving the highest yields, another group includes the nine cultures giving the lowest yields, and the remaining eighteen cultures giving medium yields comprise a third group. The positions occupied by these three groups of cultures are outlined on the diagrams of figures 1 to 3, and they correspond to the areas of high, low, and medium yields, but the yield values are omitted from the diagrams to avoid confusion. The position of any culture may readily be located on the diagram by means of its culture number, which always indicates the row and the number of the culture in the row. The rows are numbered consecutively on the left margin of each diagram, from base to apex. The culture positions in each row, represented by the intersections of the lines, are considered as numbered consecutively from left to right, the number of the last culture position in each row being given on the right margin of the diagram. The areas of high yields, corresponding to the range of the yield values for the best nine cultures, are indicated on the diagram by small crosses. The areas of low yields, corresponding to the range of the dry-weight values for the pooorest nine cultures, are marked by small circles. The position on the diagram of the culture giving the *highest* yield is marked by a larger cross, and that of the culture giving the *lowest* dry weight value is shown by a larger circle.

B. Dry weights of tops

The relations of the various salt proportions to the growth rates of the buckwheat plants during the two different developmental periods here considered, can best be compared by referring to the triangular diagrams of figure 1. The average relative dry weights of tops, as given in the third column of table 2 (series A), are here graphically represented in the upper diagram, while the lower diagram graphically represents the corresponding data of table 3 (series B). The comparisons will proceed with reference to the ranges of the high and low average dry-weight values of tops, or with respect to the extent of the corresponding areas of high and low yields as outlined on the diagrams. It is to be remembered that the position of any culture or the range of any area on these triangular diagrams, is a graphic representation of the osmotic proportions of the three salts as they occur in the solution of that culture, or of the range of these proportions in the cultures giving high or low dry weights of tops, assuming, of course, that the proportions of the salts are not altered when the solutions are introduced into the sand cultures.

1. Early period of development (series A, fig. 1). It will be observed that on the diagram of series A (fig. 1) the main area of low yields, including eight of the nine cultures embraced within the range of low (1.00–1.49) dry weights of tops, extends along the entire left margin of the diagram. Culture R_2C_7 marks the upper limit of the range of low yields. The main area of high

yields (1.95–2.38) occupies a central region on the triangle, extending to the right margin at cultures R_4C_5 and R_5C_4. This region includes eight of the nine cultures producing high yields of tops. A secondary high area is also indicated about culture R_2C_6.

The lowest yield of tops in this series occurred with culture R_1C_1 ,while the highest is shown for culture R_4C_2. The yield from this culture is 138 per cent higher than the corresponding yield from culture R_1C_1. The solution of the sand culture (R_4C_2) producing the highest dry weight of tops is characterized by having four-tenths of its total osmotic concentration due to mono-potassium phosphate, two-tenths due to calcium nitrate, and four-tenths due to magnesium sulfate. The total range of the average relative dry weights of tops for this series extends from 1.00 to 2.38.

2. Late period of development (series B, fig. 1). The average dry weights of tops for the series representing the growth period between the flowering stage and maturity, range from 1.00 to 2.30, relative to the average yield from culture R_1C_1. The diagram representing the average yields of this series shows two areas of low dry weights (1.00–1.27) on the left margin, one extending to the base and the other to the apex of the triangle. The main area of high yields, embracing eight cultures, occupies a region lying principally to the right of the vertical axis of the diagram, extending to the right margin at cultures R_5C_4 and R_3C_6, and touching the base of the triangle at culture R_1C_5. A secondary high area occurs also at the extreme lower right.

The lowest yield occurred with culture R_1C_1. The highest dry weight of tops was produced by culture R_3C_5. The solution of this culture is characterized by having three-tenths of its total osmotic concentration supplied by mono-potassium phosphate, five-tenths by calcium nitrate, and two-tenths by magnesium sulfate. The yield from this culture was 130 per cent higher than the corresponding yield from culture R_1C_1.

3. Comparison of the effects of the various salt proportions upon the growth rates during the two different developmental periods. Consideration of the relative dry weights of tops (fig. 1). From a comparison of the diagram representing the yields obtained during the early growth period (series A) with that representing the corresponding yields obtained during the late period of development (series B), it is readily apparent that there is a marked similarity between the two diagrams with respect to the positions of the areas of low top yields. Out of a total of nine cultures in each series producing low dry weights of tops, seven are included in the areas of low yields on both diagrams of figure 1. These seven cultures are included in the left marginal row, and all are characterized by low partial osmotic concentrations of calcium nitrate, but they embrace the whole range of partial concentrations of both the other salts. It thus appears that the buckwheat plants, during both the early and late developmental periods of growth, respond in much the same way to the left marginal solutions when these are supplied to the plants in a sand medium. The lowest average dry weight of tops occurred with culture R_1C_1 in both series A and series B.

Turning now to the cultures which produced high yields, it is at once apparent that there is no marked similarity between the two series with respect to the positions and ranges of the areas of high dry weights of tops as outlined on the diagrams representing the two series. Of the nine cultures in each series producing high yields, three cultures only (R_2C_5, R_4C_4, and R_5C_4) are included in the areas of high yields on both diagrams. Culture R_4C_2 in series A, and R_3C_5 in series B, each producing the highest yield of tops in its respective series, are shown to occupy positions on opposite sides of the diagrams. The osmotic proportions of the three salts characterizing the former are markedly different from those which characterize the latter. The maximum yield of tops was produced during the early period of development in a sand medium provided with a solution having a higher osmotic proportion of mono-potassium phosphate, a much lower proportion of calcium nitrate, and a much higher one of magnesium sulfate than had the solution in the sand culture which gave the highest yield of tops during the late developmental period (series B). Thus, with respect to the groups of cultures producing high yields of tops, it is at once clear that the response of the plants to the proportions of the three salts in the various solutions supplied to the sand cultures, is markedly different during the two different developmental growth periods represented by series A and series B.

The readiness with which the older plants of series B respond to the variations in the proportions of the salts in the different solutions, is brought out by a comparison of the total ranges of the average relative top yields of the two series. The variations in the average relative yield values for series A extend from 1.00 to 2.38, giving a total range in these values of 1.38 from the lowest to the highest. The corresponding yield values of series B vary from 1.00 to 2.30, showing a total range of 1.30, from the lowest to the highest value. It will thus be observed that the highest yield value for each of the two series is more than double that of the lowest.

It is interesting here to compare the salt proportions producing the highest yields and the lowest yields in the sand cultures of the present study with those giving the best and the poorest yields in the corresponding series of solution cultures previously carried out. A comparison of the best sand culture of the series conducted during the early period of development (series A) with the best solution culture of the corresponding series, brings out the fact that the salt proportions of these two cultures are the same. These are the salt proportions of culture R_4C_2, the highest yield of tops occurring with this culture in each of the two corresponding series in question. The poorest sand culture and the poorest solution culture in the corresponding series carried out during the early growth period, show a marked difference in the proportions of the three salts. The former has the salt proportions of culture R_1C_1, while the latter is characterized by those of culture R_1C_4. A similar comparison of the best and the poorest physiological balance of salt proportions for the growth of buckwheat tops in sand cultures and in solution cultures carried out during

the late period of development (series B) shows that the two series agree in the proportions of the three salts required for the best yields. They agree also in the salt proportions giving the poorest dry weights of tops. In each of these two series the highest yield occurred with the salt proportions of culture R_3C_5, and the poorest yield with those of culture R_1C_1. It thus appears that the physiological properties of the nutrient solutions giving the highest yields of buckwheat tops are not greatly disturbed by the introduction of these solutions into the sand cultures, when these properties are judged by their relative effects upon the plants grown in the solutions and in sand cultures provided with the solutions. This is clearly indicated by the perfect agreement between the salt proportions characterizing the cultures giving maximum yields in the corresponding series of sand and solution cultures. Agreements similar to those here pointed out are indicated for other salt proportions characterizing cultures which produced high yields, as well as some which gave low yields, in corresponding series of sand and solution cultures, but these can best be brought out by a comparison of the triangular diagrams representing the series in question.

4. Comparison of the ion ratio values and the ranges of these for high and for low yields of tops. The cation ratio values and the ranges of these for the cultures giving the best nine and the poorest nine yields of buckwheat tops for each of the two series here considered, are presented in table 4. The table is divided into two vertical sections. The first column in each section gives the culture numbers and this is followed by three columns giving the ion ratio values and the total ranges of these for the best and the poorest nine cultures of the series indicated at the top. The cultures are arranged in the descending order of the magnitudes of the Mg/Ca ratio. At the bottom of the table are given the maximum and minimum values and the total range of these for the entire series. The ratio values of the culture giving the highest yield in each series are indicated in black-face type, while those of the culture giving the lowest dry weight in each series appear in italics. The cultures included in table 4 are comprised in the areas of high and of low yields outlined on the triangular diagram of figure 1.

From a comparison of the ion ratio values for the group of cultures producing high yields of tops in series A, with those of the corresponding group of series B, as given in table 4, it is at once evident that there is no substantial agreement between the two series with respect to the ranges in the values of any of the three cation ratios, Mg/Ca, Mg/K, and Ca/K. In series A the group of cultures giving high yields is charasterized by a relatively narrow range in the magnitudes of each of the three ratios. The ratio ranges of this group of cultures are restricted to the lower one-third of the corresponding total ranges of the entire series. Series B, on the other hand, shows a low range (0.24 to 1.54) in the values of the Mg/Ca ratio, a medium range (0.28 to 5.55) for the ratio Mg/K, and a relatively wide range (0.58 to 5.76) in the values of the ratio Ca/K. During the early period of development, therefore, good growth of

tops was associated with Mg/Ca ratio values between 0.38 and 4.81; with Mg/K ratio values between 0.28 and 3.47; and with values of the Ca/K ratio between 0.36 and 2.16. During the late period of development good growth of tops occurred with ranges in the ratio values as follows: Mg/Ca between 0.24 and 1.54; Mg/K between 0.28 and 5.55; and Ca/K between 0.58 and 5.76.

The highest yielding cultures, R_4C_2 in series A, and R_3C_5 in series B, agree

TABLE 4

Cation ratio values and ranges of these values for cultures producing high and low yields (best nine and poorest nine cultures, respectively) of buckwheat tops during the early and the late developmental periods

	SERIES A (FIRST 4-WEEK GROWTH PERIOD)				SERIES B (SECOND 4-WEEK GROWTH PERIOD)			
	Culture number	Mg/Ca	Mg/K	Ca/K	Culture number	Mg/Ca	Mg/K	Ca/K
High yields	R_3C_2	4.81	2.32	0.48	R_1C_5	1.54	5.55	3.60
	R_4C_2	**3.85**	**1.39**	**0.36**	R_3C_4	1.44	1.39	0.96
	R_2C_3	3.21	3.47	1.08	R_2C_5	1.15	2.08	1.80
	R_4C_3	1.92	1.04	0.54	R_4C_4	0.96	0.69	0.72
	R_5C_3	1.28	0.56	0.43	R_3C_5	**0.77**	**0.93**	**1.20**
	R_4C_4	0.96	0.69	0.72	R_2C_6	0.64	1.39	2.16
	R_2C_6	0.64	1.39	2.16	R_5C_4	0.48	0.28	0.58
	R_5C_4	0.48	0.28	0.58	R_3C_6	0.32	0.46	1.44
	R_4C_5	0.38	0.35	0.90	R_1C_8	0.24	1.39	5.76
Range		4.43	3.19	1.80		1.30	5.27	5.18
Low yields	R_1C_1	*15.40*	*11.10*	*0.72*	R_1C_1	*15.40*	*11.10*	*0.72*
	R_2C_1	13.46	4.86	0.36	R_2C_1	13.46	4.86	0.36
	R_3C_1	11.55	2.78	0.24	R_4C_1	9.61	1.74	0.18
	R_4C_1	9.61	1.74	0.18	R_5C_1	7.70	1.11	0.14
	R_5C_1	7.70	1.11	0.14	R_1C_2	6.74	9.72	1.44
	R_6C_1	5.77	0.69	0.12	R_2C_2	5.77	4.17	0.72
	R_7C_1	3.85	0.40	0.10	R_6C_1	5.77	0.69	0.12
	R_8C_1	1.92	0.18	0.09	R_7C_1	3.85	0.40	0.10
	R_2C_7	0.27	0.69	2.52	R_8C_1	1.92	0.18	0.09
Range		15.13	10.92	2.43		13.48	10.92	1.35
Entire series	Maximum	15.40	11.10	5.76				
	Minimum	0.24	0.18	0.09				
	Range	15.16	10.92	5.67				

in showing relatively low values for all three ratios, although there is considerable difference in the corresponding ratio values of the two cultures. The values of the three ratios Mg/Ca, Mg/K, and Ca/K for the culture giving the highest yield of tops in series A are 3.85, 1.39, and 0.36, respectively, and the corresponding values for the culture producing the highest yield of tops in series B are 0.77, 0.93, and 1.20, respectively.

A similar comparison of the ranges in ratio values for the group of lowest-yielding cultures in series A with those of the corresponding group in series B, brings out the fact that these two groups agree in showing very wide ranges in the values of the two ratios Mg/K and Mg/Ca, and a relatively narrow range in the values of the ratio Ca/K. Each of the two groups embraces the entire range of the values of the Mg/K ratio. Each group also includes the highest value of the Mg/Ca ratio and the lowest value of the ratio Ca/K.

The two series agree in showing the lowest yield for the same culture, R_1C_1. This culture is characterized by the highest value of the ratios Mg/Ca (15.40) and Mg/K (11.10), and by a relatively low value of the ratio Ca/K (0.72).

From a consideration of these data it appears that the relation between the growth rates and the ion ratio values is markedly different for the group of cultures producing high yields during the early developmental periods and the group giving corresponding yields during the late period of development. On the other hand, there is a striking similarity, with respect to this relation, between the group of low-yielding cultures of series A and that of series B.

C. Dry weights of roots.

The average relative dry weights of roots as given in the last column of table 2, for series A, and in the fifth column of table 3 for series B, are represented graphically on the diagrams of figure 2. The upper diagram represents the data of the root yields of the young plants obtained at the flowering stage, while the lower diagram represents the corresponding data of the yields of the older plants, obtained at maturity. These two diagrams, like those of figure 1, representing the average relative yields of tops, will be compared with reference to the ranges of the yield values of the best nine and of the poorest nine cultures, and also with respect to the positions and extent of the corresponding areas of high and low yield values as these are outlined on the diagrams.

1. Early period of development (series A, fig. 2). On the diagram of series A (fig. 1), the low yields (0.73–1.06) are represented as occupying three areas bordering on the left margin of the diagram. Cultures R_1C_2 and R_1C_8 each mark the upper limit in the ranges of low-yield values. Both cultures are, therefore, included in the group of cultures giving low yields. The main area of high yields (1.41–1.65), occupies a central region on the diagram, extending to the right margin at cultures R_4C_5 and R_5C_4. This area includes eight of the group of nine cultures producing high yields. A secondary high area is indicated about culture R_6C_2.

The lowest yield of buckwheat roots in this series occurred with culture R_8C_1. The highest yield is shown for culture R_5C_4. The yield from this culture is 65 per cent higher than the corresponding yield from culture R_1C_1. The solution furnished to the sand medium of this culture is characterized by having five-tenths of its total osmotic concentration due to mono-potassium

phosphate, four-tenths due to calcium nitrate, and one-tenth to magnesium sulfate. The total range in the values of the average relative dry weights of roots of this series extends from 0.73 to 1.65.

2. Late period of development (series B, fig. 2). The main area of low (0.78–1.07) average yields represented on the diagram of this series occupies a region bordering on the lower left margin and extends to the base of the triangle. Another low area, including three cultures, borders on the upper right margin and extends to the apex of the diagram. A small low area is also indicated about culture R_1C_6. The high (1.57–1.88) average yields of roots are represented on the diagram by a single area occupying a central region mainly to the right of the vertical axis of the diagram, and extending to the right margin at culture R_3C_6.

The lowest average yield of roots in this series was produced by culture R_6C_3. The highest yield occurred with culture R_3C_5, and was 88 per cent higher higher than the corresponding yield from culture R_1C_1. The solution supplied to the sand of the highest-yielding culture derived three-tenths of its total osmotic concentration from mono-potassium phosphate, five-tenths from calcium nitrate, and two-tenths from magnesium sulfate. The total range in the yield values obtained from the cultures of this series extends from 0.78 to 1.88, relative to the yield from culture R_1C_1.

3. Comparison of the effects of the various salt proportions upon the growth rates during the two different developmental periods. Consideration of the relative dry weights of roots (fig. 2.) A comparison of the two diagrams of figure 2, representing the yield data obtained from the young plants at the flowering stage (series A), and the corresponding data obtained from the mature plants (series B), shows the agreements and the disagreements between the two series, with respect to the distribution of the areas of low yields, to be nearly equally divided. The area of low yields at the lower left of the diagram of series A has a somewhat corresponding area on the diagram of series B. The four cultures R_1C_1, R_2C_1, R_3C_1, and R_8C_1 are included in the areas of low yields of roots on both diagrams. With respect to the remaining areas of low yields, the two diagrams show no similarity. The lowest yield of roots in series A occurred with culture R_8C_1, while series B shows the lowest yield for culture R_6C_3.

The two diagrams of figure 2 show a certain degree of similarity with respect to the areas representing high average yields of roots. This is indicated by the fact that six of the nine highest-yielding cultures in series A appear also in the area of high root yields on the diagram of series B. The highest yields of roots, however, are shown for different cultures in the two series. The highest yield in series A occurred with culture R_5C_4. This culture is indicated as producing a low medium yield in series B. The highest yield of roots in series B is shown for culture R_3C_5. Thus the maximum yield of roots was produced during the early period of development in a sand medium provided with a solution having a higher osmotic proportion of mono-potassium phos-

phate and a lower proportion of both calcium nitrate and magnesium sulfate than had the solution in the sand culture giving the highest yield of roots during the late period of development. While it has been shown that there is a certain degree of similarity between the areas of high yields on the diagrams representing the relative dry weights of roots of series A and of series B, it is evident that there is considerable difference in the manner in which the roots of the two series responded to the variations in the proportions of the three salts. This is clearly indicated by the fact that neither the lowest nor the highest dry weights of roots occurred with corresponding cultures of the two series.

It has already been shown by a comparison of the total ranges of the average relative values of the top yields of the two series, that the older plants of series B responded quite as readily to the variations in the proportions of the salts in the solutions of the different cultures as did the young plants of series A. This is emphasized also by a similar comparison of the total ranges in the values of the relative dry weights of roots of the two series. The variations in the relative yield values of the roots of series A extend from 0.73 to 1.65, showing a total range of 0.92. The corresponding yield values of series B vary from 0.78 to 1.88, giving a total range of 1.10 from the lowest to the highest value. It will thus be observed that the range in the yield values of series B is somewhat higher than that of series A. The highest yield value for each of the two series is more than double that of the lowest, which is true also in the case of top yields.

A comparison of the diagrams of figure 2 with the corresponding ones of figure 1, brings out some interesting correlations between the growth of tops and of roots. Thus, five of the nine cultures giving high yields of tops in series A are included also in the areas of high root yields, and six cultures included in areas of low top yields appear also in the areas of low root yields. In this series, however, the *highest* yield of tops and of roots occurred with different cultures, as did also the *lowest* yield of tops and of roots.

On the diagram representing the relative yields obtained at the end of the late developmental period (series B), the main area of high top yields and that of high root yields are in very good agreement. In this series the highest yield of tops and of roots occurred with the same culture, R_3C_5. Five cultures giving low top yields also produced low yields of roots. But the lowest dry weights of tops and of roots are shown for different cultures.

4. Comparison of the ion ratio values and the ranges of these for high and low yields of roots. Table 5 presents the cation ratio values and the ranges of these values for the cultures giving the best nine and the poorest nine yields of buckwheat roots for each of the two periods of development here considered. This table conforms in every respect to table 4. Inspection of table 5 brings out the fact that the two series agree in showing a relatively low range in the values of each of the three cation ratios for the group of cultures in both series producing high root yields. The ratio ranges for this group of cultures in

both series are restricted to the lower one-third of the corresponding total ranges for the entire series. These ranges lie near, but do not include, the lowest values of the respective ratios occurring in the entire series. From this it appears that good growth of roots during each of the two periods of development was associated with low values of all three cation ratios. It will be observed, however, that there is considerable difference between the range values

TABLE 5

Ion ratio values and ranges of these values for cultures producing high and low yields (best nine and poorest nine cultures, respectively) of buckwheat roots during the early and the late developmental periods

	SERIES A (FIRST 4-WEEK GROWTH PERIOD)				SERIES B (SECOND 4-WEEK GROWTH PERIOD)			
	Culture number	Mg/Ca	Mg/K	Ca/K	Culture number	Mg/Ca	Mg/K	Ca/K
High yields	R_3C_3	2.56	1.85	0.72	R_2C_5	1.15	2.08	1.80
	R_4C_3	1.92	1.04	0.54	R_3C_3	2.56	1.85	0.72
	R_6C_2	1.92	0.46	0.24	R_6C_2	1.92	0.46	0.24
	R_3C_4	1.44	1.39	0.96	R_3C_4	1.44	1.39	0.96
	R_4C_4	0.96	0.69	0.72	R_5C_3	1.28	0.56	0.43
	R_3C_5	0.77	0.93	1.20	R_2C_6	0.64	1.39	2.16
	R_2C_6	0.64	1.39	2.16	R_4C_4	0.96	0.69	0.72
	R_5C_4	**0.48**	**0.28**	**0.58**	R_3C_5	**0.77**	**0.93**	**1.20**
	R_4C_5	0.38	0.35	0.90	R_3C_6	0.32	0.46	1.44
Range		2.18	1.57	1.92		2.56	3.71	1.92
Low yields	R_1C_1	15.40	11.10	0.72	R_1C_1	15.40	11.10	0.72
	R_2C_1	13.46	4.86	0.36	R_2C_1	13.46	4.86	0.36
	R_3C_1	11.55	2.78	0.24	R_3C_1	11.55	2.78	0.24
	R_5C_1	7.70	1.11	0.14	R_2C_2	5.77	4.17	0.72
	R_1C_2	6.74	9.72	1.44	R_3C_2	4.81	2.32	0.48
	R_1C_3	3.85	8.34	2.16	R_8C_1	1.92	0.18	0.09
	R_7C_1	3.85	0.40	0.10	R_1C_6	0.96	4.17	4.32
	R_8C_1	*1.92*	*0.18*	*0.09*	R_7C_2	0.96	0.20	0.20
	R_1C_8	0.24	1.39	5.76	R_6C_3	*0.64*	*0.23*	*0.36*
Range		15.16	10.92	5.67		14.76	10.92	4.23
Entire series	Maximum	15.40	11.10	5.76				
	Minimum	0.24	0.18	0.09				
	Range	15.16	10.92	5.67				

of the ratios Mg/Ca and Mg/K in the two series, the range in the values of these two ratios for the group of cultures producing high root yields being 2.18 and 1.75, respectively, in series A, and 2.56 and 3.71, respectively, in series B. The range value of the Ca/K ratio for the group of high-yielding cultures in each of the two series is 1.92.

The cultures R_5C_4 and R_3C_5, giving the highest yields in series A and B, respectively, agree in showing low values for the cation ratios characterizing

these cultures, but like the ratio ranges characterizing the groups of high-yielding cultures in the two series, the values of the corresponding ratios for the two cultures show considerable variations. Thus, the values of the three ratios Mg/Ca, Mg/K, and Ca/K for the culture (R_5C_4) giving the highest yield in series A, are 0.48, 0.28, and 0.58, respectively, and the corresponding ratio values for the culture (R_3C_5) producing the highest dry weight of roots in series B are 0.77, 0.93, and 1.20.

The group of nine cultures producing low yields of roots during the early period of development is characterized by the highest and lowest values of each of these cation ratios. The ranges in the magnitudes of these cation ratio values for this group of cultures are, therefore, coextensive with the corresponding ranges for the entire series. The group of nine low-yielding cultures for the late developmental period also shows very wide ranges in the values of the ratios Mg/Ca, and Ca/K, these ranges being 14.76 and 4.23, respectively. It embraces also the full range of the values of the Mg/K ratio.

From a study of the ion ratio data for high and for low yields of tops and of roots as given in tables 4 and 5, respectively, it appears that high yields of tops and of roots are, in general, associated with relatively low, but not the lowest, values of the three cation ratios. Low yields of tops and of roots, on the other hand, generally occur with solutions which are characterized by values of one or more of the three cation ratios which are either relatively high or relatively very low

D. Dry weights of seeds

The actual and the relative dry weights of seeds are presented in table 2 in connection with the corresponding data for tops and roots of the same series. The actual dry-weight values of seeds, as given in the table, represent the average yields from two similar series. The relative yield values were obtained in the same manner as were those of tops and of roots. The last column of table 2 gives the ratio values obtained by dividing the average actual dry weight values of tops by the corresponding values of seeds. These ratio values represent the yields of tops expressed in terms of the corresponding yields of seeds considered as 1.00.

The relative yields of seeds are graphically represented on the triangular diagram of figure 3, which corresponds to those of figures 1 and 2, representing in the same manner the relative yields of tops and of roots, respectively. It will be observed that the main area of low yields of seeds, including five of the nine cultures embraced within the range of low (0.71–0.92) dry weights, occupies a region bordering on the left margin of the diagram. Three smaller outlying areas of low yields also are indicated. The total range (1.31–1.68) of high yields of seeds is represented on the diagram by a single area occupying a central region at the base of the triangle and extending upward to the center, and to the right margin at culture R_3C_6.

The lowest yield of seeds occurred with culture R_3C_1, and the highest dry weight was produced by culture R_3C_5. The average yield from this culture was 68 per cent higher than the corresponding yield from culture R_1C_1. The total range of the average dry weights of seeds extended from 0.71 to 1.68.

A comparison of the diagram of figure 3 with those representing the yields of tops and of roots of the same series (series B, fig. 1 and 2, respectively), shows a marked degree of similarity between the diagrams with respect to the distribution of the areas of high and also of low yields. Five of the nine cultures shown in the area of high yields of seed are included in the area of high top yields, and four are also included in the area of high yields of roots. The maximum yields of tops, of roots, and of seeds were produced by the same culture, R_3C_5, but no such correlation is shown for minimum yields. The diagrams representing the three kinds of yields agree, however, in showing the main areas of low dry weights to occupy somewhat corresponding regions on the left margins of the diagrams. It thus appears that the yields of tops, of roots, and of seeds vary in a somewhat similar manner with respect to the variations in the proportions of the three salts in the solutions supplied to the sand cultures. The plants of each of the 36 cultures of this series produced an abundance of large, fairly uniform, and well filled seeds. From an inspection of the last column of table 1, giving the yields of tops in terms of the corresponding yields of seeds, considered as unity, it will be observed that these ratio values for eight cultures lie between 3.0 and 4.0, the values for twenty-three cultures lie between 2.0 and 3.0, and for five cultures the values of the ratios are between 1.0 and 2.0. The ratio of the average yield of tops to the average yield of seeds for the entire series is 2.27. This indicates that the average yield of tops for this series is only 2.27 times the corresponding yield of seeds.

A comparison of the diagram of figures 3 with the diagram graphically representing the yields of seeds from the corresponding series of solution cultures previously carried out, shows that the two corresponding diagrams are in partial agreement with respect to the distribution of the areas of high and low yields of seeds. Five of the nine high-yielding cultures in the present sand culture series are also indicated as producing high dry weights of seeds in the solution cultures, but the maximum yields of seeds did not occur with corresponding cultures of the two series. The highest yield of seeds in the sand cultures occurred with culture R_3C_5, which produced a medium yield in solution culture. Culture R_3C_3, which gave the maximum yield of seeds in solution culture, produced a medium yield in the present series of sand cultures. It is to be noted also that culture R_4C_3, which gave a high yield of seeds in sand culture, produced a low yield in solution culture, while culture R_5C_4, which is indicated as producing a high yield in solution culture, gave a low yield in sand culture. It may be said, however, that the two series show a marked similarity with respect to the position on the triangular diagrams occupied by the main areas of high and of low yields of seeds.

II. Effect of the sand medium upon the physiological properties of the solutions

As previously stated, the maximum yields of buckwheat tops obtained during the early developmental period, from the present series of sand cultures, and from the corresponding series of solution cultures previously carried out, were produced by the same set of salt proportions. These were the salt proportions of culture R_4C_2. The corresponding series of sand and solution cultures conducted during the late period of development also agreed in showing their highest yields of tops for the same set of salt proportions, these being the salt proportions characterizing the culture R_3C_5. It thus appears that the physiological properties of the solutions producing maximum yields of buckwheat tops are not altered to any great extent when these solutions are introduced into the sand here employed, and in the manner described.

It is possible, of course, that solutions such as were here employed may undergo, not only a reduction in the total concentration, but also a change in the relative proportions of the constituent salts and ions, as the result of contact with the solid sand particles. It should again be emphasized, however, that in the present experiments, the sand of each culture was flooded with the nutrient solution, which was then drawn off, leaving the culture with a fixed solution content (15 per cent of the weight of the air-dry sand), after which two more portions of the solution (250 cc. to each portion) were passed through the sand cultures before the culture pots were sealed and the time period of the experiment actually begun. With such treatment and with subsequent renewal of the solutions every third day, it appears reasonable to suppose that equilibrium would soon become established with respect to the adsorptive capacity of the sand, after which the solution should suffer no further alteration from this factor, either in total concentration or in the relative proportions of the salts and ions, excepting as the adsorptive action of the sand might change with changes in temperature.

An attempt was here made to determine the influence of the sand upon the total concentration of the various solutions in the sand cultures employed, both at the beginning and at the end of an experimental period of 4 weeks' duration. For these tests the cryoscopic method was employed. A series of 36 sand cultures was prepared as already described. After the seedlings had been transplanted to the culture pots, the sand cultures reduced to the desired moisture content (15 per cent on the air-dry weight basis), and the culture pots sealed, these were allowed to stand in the greenhouse for 24 hours. A sufficient quantity of solution was then withdrawn from each sand culture, by the method already described, to be tested for the lowering of the freezing point. The solution withdrawn from each culture was replaced by an equal quantity of new solution. The cultures were then continued with renewal of solutions every third or fourth day. At the end of the growth period the solutions in the sand cultures were renewed in the usual way and the cultures were again allowed to stand for 24 hours. The cultures were then weighed

and the water lost by transpiration during the preceding 24 hours was restored by the addition of distilled water. After an interval of from 20 to 30 minutes to allow the water films to come into partial equilibrium, a small quantity of solution sufficient for the test of the lowering of the freezing point was withdrawn from each sand culture, after which the plants were harvested in the usual manner.

In table 6 are presented the results of the freezing-point determinations of the solutions withdrawn from the sand cultures supplied with the different solutions of the optimal 3-salt series employed in these studies. The solution or culture numbers referring to the triangular diagram are given in the first column of the table, which is divided into two vertical sections of three columns each. The first section presents the actual depressions of the freezing point (after corrections were made for undercooling), the osmotic concentration value at 25°C., and the variations from the original calculated osmotic concentration value of 1.75 atmospheres, for each of the 36 different solutions tested at the beginning of the growth period. The last section gives the corresponding data for the tests made at the end of the growth period. Each of the data in this table represents the average of two or more tests.

It will be observed that the osmotic-concentration values of the solutions extracted from the sand cultures at the beginning of the growth period are, with the single exception of solution R_7C_1, in very close agreement with the calculated value of the original solutions. The greatest deviation above 1.75 atmospheres is 13.15 per cent (solution from culture R_7C_1), and the greatest below this calculated value is 4.75 per cent. The average osmotic concentration value for the entire series is 1.753 atmospheres, which represents a deviation from the original value of only 0.17 per cent. The results of the tests made at the end of the growth period show a somewhat wider variation from the original concentration value than do those of the tests made at the beginning of the growth period, the greatest deviation above the concentration value of the original solutions being 9.14 per cent, while the greatest deviation below this value is 14.28 per cent. However, the osmotic concentration values of these solutions (with the exception, perhaps, of those from cultures R_2C_4, R_3C_2, R_7C_1, and R_7C_2) show as close agreement with the calculated value of the original solutions as might be expected, considering the numerous manipulations involved in the repeated renewal of solutions during a period of 4 weeks, and the chance of cumulative slight errors resulting therefrom. The average osmotic concentration value for the entire series is 1.72 atmospheres which represents a minus deviation from the calculated value of only 1.71 per cent. The majority of the solutions of this series show minus deviations from the calculated value, but many also show plus deviations, so that little significance can be attached to the comparatively slight deviations from the calculated concentration value, especially since there is no regularity in the manner in which the deviations occur.

Variations in the total concentrations such as were here observed, are scarcely sufficient to produce any marked changes in the growth rates, especially since these concentrations lie well within the range of optimal growth for these

TABLE 6

Concentration data of solutions extracted from sand cultures at the beginning and at the end of a 4-week growth period

CULTURE NUMBER	BEGINNING OF GROWTH PERIOD			END OF GROWTH PERIOD		
	Depression of the freezing point	Osmotic-concentration value at 25°C.	Variation from calculated osmotic-concentration value (1.75 atm.)	Depression of the freezing point	Osmotic-concentration value at 25°C.	Variation from calculated osmotic-concentration value (1.75 atm.)
	°C.	atm.	per cent	°C.	atm.	per cent
R_1C_1	0.134	1.76	0.57	0.135	1.77	1.14
C_2	0.132	1.74	−0.57	0.132	1.74	−0.57
C_3	0.130	1.72	−1.71	0.132	1.74	−0.57
C_4	0.133	1.75	0.00	0.140	1.84	5.14
C_5	0.134	1.76	0.57	0.123	1.62	−7.43
C_6	0.131	1.72	−1.71	0.140	1.84	5.14
C_7	0.131	1.72	−1.71	0.123	1.62	−7.43
C_8	0.132	1.74	−0.57	0.142	1.86	6.28
R_2C_1	0.133	1.75	0.00	0.138	1.82	4.00
C_2	0.126	1.67	−4.57	0.140	1.84	5.14
C_3	0.134	1.76	0.57	0.140	1.84	5.14
C_4	0.130	1.72	−1.71	0.145	1.91	9.14
C_5	0.133	1.75	0.00	0.132	1.74	−0.57
C_6	0.135	1.77	1.14	0.126	1.67	−4.57
C_7	0.134	1.76	0.57	0.132	1.74	−0.57
R_3C_1	0.134	1.76	0.57	0.135	1.78	1.71
C_2	0.136	1.78	1.71	0.116	1.53	−12.58
C_3	0.142	1.86	6.28	0.126	1.67	−4.57
C_4	0.136	1.79	2.29	0.126	1.67	−4.57
C_5	0.133	1.75	0.00	0.130	1.72	−1.71
C_6	0.130	1.72	−1.71	0.121	1.60	−8.58
R_4C_1	0.133	1.75	0.00	0.122	1.61	−8.28
C_2	0.131	1.72	−1.71	0.132	1.74	−0.57
C_3	0.137	1.80	2.86	0.133	1.75	0.00
C_4	0.144	1.89	8.00	0.132	1.74	−0.57
C_5	0.131	1.72	−1.71	0.121	1.60	−8.58
R_5C_1	0.134	1.76	0.57	0.133	1.75	0.00
C_2	0.132	1.74	−0.57	0.132	1.74	−0.57
C_3	0.135	1.77	1.14	0.132	1.74	−0.57
C_4	0.132	1.74	−0.57	0.135	1.78	1.71
R_6C_1	0.132	1.74	−0.57	0.124	1.63	−6.86
C_2	0.133	1.75	0.00	0.140	1.84	5.14
C_3	0.133	1.75	0.00	0.129	1.70	−2.86
R_7C_1	0.151	1.98	13.15	0.114	1.50	−14.28
C_2	0.133	1.75	0.00	0.114	1.50	−14.28
R_8C_1	0.129	1.70	−2.86	0.129	1.70	−2.86

plants, so that the results of these tests are in entire accord with the behavior of the plants in solution cultures and in the corresponding sand cultures, as this behavior was judged by the criterion of dry-top yields.

III. Transpiration and water requirement

Since transpiration may be regarded as a valuable indicator of the general health and vigor of plants, it was deemed worth while to compare the relative amounts of water lost from the various cultures during their growth period with the data of the other plant measurements. From the relation between the amounts of water lost by transpiration and the dry-weight yields of tops, roots, and seeds, may be derived also the ratios representing the amount of water lost for each single gram of dry plant substance produced. These ratios of transpiration to yields represent the water requirements of the plants. Because of the importance of this criterion of plant growth, and for the sake of completeness, it seemed desirable also to compare these derived data with the direct quantitative plant measurements.

The data of transpiration and water requirements are presented in table 7 in three sections. The first section gives the relative amounts of water loss from the cultures of the two series representing the two different developmental periods here considered. This is followed by the section giving the water requirments of tops and of roots for series A. The last section presents the corresponding data for series B, and also the water requirements of seeds for this series. The various data for each culture are expressed in terms of the corresponding data for culture R_1C_1 in the respective columns. In each column the actual value for this culture is given in parentheses just below the relative value.

The average data of table 7 were plotted on triangular diagrams in the same manner as were the yields of tops, roots, and seeds, and the corresponding diagrams of the two different series thus obtained were compared with each other and also with the corresponding yield diagrams already given (figs. 1, 2 and 3). The various diagrams graphically representing the data of table 7 are not here presented, but the main points of interest brought out by these comparisons will be given in brief.

A. Relation of transpiration to yields

The main areas representing high and low water loss on the transpiration diagram of series A occupy positions corresponding very closely with those occupied by the main areas of high and low yields, respectively, of tops and of roots on the yield diagrams. The highest total amount of water loss from a single culture of this series, however, did not occur with the same culture giving the maximum yield of tops, nor with that producing the highest yield of roots. In series B the agreement between the transpiration diagram and the corresponding yield diagrams of tops and of roots, with respect to the

main areas of both high and low yields, is even more pronounced than it is in series A. The maximum yield of tops and of roots and the greatest amount

TABLE 7

Data of transpiration and water requirement; series A grown to the flowering stage, series B grown from the flowering stage to maturity in sand cultures supplied with 3-salt solutions

CULTURE NUMBER	TRANSPIRATION		WATER REQUIREMENT				
			Series A		Series B		
	Series A	Series B	Tops	Roots	Tops	Roots	Seeds
R_1C_1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	(502)	(2384)	(254)	(1716)	(485)	(4599)	(794)
C_2	1.21	1.59	0.83	1.21	1.25	1.45	1.32
C_3	1.31	1.68	0.89	1.50	1.10	1.01	1.23
C_4	1.36	1.81	0.83	1.26	1.07	1.17	1.16
C_5	1.42	1.81	0.89	1.29	0.98	1.12	1.21
C_6	1.49	1.64	0.90	1.28	1.04	1.22	1.50
C_7	1.40	1.60	0.77	1.26	0.93	0.98	1.66
C_8	1.35	1.66	0.91	1.66	0.89	1.22	2.04
R_2C_1	1.06	1.49	1.00	1.35	1.13	0.97	1.96
C_2	1.53	1.32	0.85	1.19	1.01	1.15	2.14
C_3	1.59	1.62	0.86	1.35	0.95	0.90	2.17
C_4	1.41	1.73	0.80	1.32	0.93	0.86	1.41
C_5	1.68	1.89	0.92	1.26	0.99	0.85	1.38
C_6	1.86	1.79	0.93	1.45	0.93	0.91	1.36
C_7	1.48	1.59	1.04	1.35	0.89	1.05	1.44
R_3C_1	1.26	1.60	1.01	1.30	0.96	1.20	2.98
C_2	1.49	1.86	0.89	1.39	1.01	1.65	1.61
C_3	1.62	1.38	0.88	1.10	0.73	0.62	1.40
C_4	1.59	1.72	0.89	1.42	0.85	0.85	1.25
C_5	1.55	2.03	0.86	1.43	0.78	0.78	1.17
C_6	1.49	1.84	0.93	1.36	0.94	0.89	1.26
R_4C_1	1.39	1.46	0.98	1.16	1.04	1.09	1.90
C_2	1.72	1.77	0.76	1.33	1.07	0.88	1.40
C_3	1.61	1.83	0.80	1.26	1.03	0.99	1.35
C_4	1.71	1.81	0.89	1.23	0.88	0.77	1.77
C_5	1.69	1.61	0.89	1.28	1.15	1.06	1.98
R_5C_1	1.26	1.69	1.10	1.55	1.47	0.96	1.40
C_2	1.33	1.77	0.89	1.35	1.11	0.87	1.55
C_3	1.74	1.85	0.87	1.63	1.01	0.87	1.80
C_4	1.50	1.61	0.83	0.99	0.85	0.94	1.78
R_6C_1	1.21	1.44	0.99	1.20	1.26	0.93	2.07
C_2	1.48	1.71	0.84	1.07	1.04	0.88	1.55
C_3	1.51	1.75	0.85	1.23	1.04	1.61	1.64
R_7C_1	1.20	1.64	0.97	1.22	1.27	1.32	1.54
C_2	1.34	1.64	0.90	1.26	1.09	1.06	1.59
R_8C_1	1.24	1.65	0.95	1.26	1.32	1.30	1.70
K	1.50	1.76	0.92	1.36	0.97	0.85	1.30
T	1.64	1.72	0.91	1.23	1.01	1.05	2.03

of transpirational water loss from any single culture here occurred with the same culture, R_3C_5. The lowest yield of tops and the smallest amount of water loss is shown for culture R_1C_1.

From these considerations it is clear that high transpiration, in general, is associated with high yields of tops and of roots, and low transpiration with low yields, an observation which is in entire accord with what has already been found in the study of buckwheat in solution cultures corresponding to the present study of these plants in sand cultures.

A comparison of the transpiration diagram of series A with the corresponding diagram of series B, brings out the fact that there is no agreement between the two diagrams with respect to the areas of high transpiration values. The two series agree, however, in showing the main regions of low transpiration as bordering on the left margins of the diagrams. It thus appears that the relation between the various salt proportions and transpiration, with respect to the two different periods of development, is much the same as is the relation between the salt proportions and the yields of tops and of roots.

B. Relation of water requirements to yields

A comparison of the water-requirement diagrams with the corresponding yield diagrams, shows that the relations between water requirement of tops and the dry-weight yields of tops are very well defined in each of the two series here considered. On the diagram representing the water requirements of tops of series A, the areas of high values agree absolutely with the areas of low values on the corresponding diagram of top yields, while the regions of low water requirements agree in a general way with those of high top yields. There is no detailed relation, however, between the water requirements of roots and root yields in this series.

On the diagrams of series B, the regions of low water requirements correspond very closely with those of high top yields, while the areas of high water requirements and low top yields show equally good agreement. There is a marked tendency also toward the same relations between the water requirements of roots and root yields in this series, although the agreements are not so exact as they are in the case of the water requirements of tops and top yields.

A comparison of the water requirement diagram for seeds with the corresponding yield diagram shows the areas representing low water requirements to correspond very closely with those of high seed yield, while the regions of high water requirements of seeds are in close agreement with those of low seed yields. Thus, with the sand cultures of the present study, as with the corresponding solution cultures of the earlier work, low water requirements are associated with high yields of tops, roots, and seeds, and high water requirements correspond to low yields.

From a comparison of the corresponding diagrams representing the water

requirements of the two different series, it is clear that there is as little correlation between the water requirements of the young plants of series A and the older ones of series B as there is between the dry-weight yields, either of tops or of roots, of the two different series, or between the transpirational water loss from the cultures of series A and those of series B. Thus, by whatever set of measurements the relation between the growth rates and the proportions of the salts in the media is judged, this relation is found to be markedly different for the two different developmental periods.

In the earlier work with solution cultures corresponding to the present series of sand cultures, it was emphasized that the changes in the physiological requirements of these plants, with respect to the proportions of the salts in the nutritive media might be a gradual process extending over a comparatively long interval of time, involving perhaps the entire life period of the plants. On the other hand, it was pointed out that the change in salt requirements of the plants may take place comparatively rapidly with the marked changes which occur within the plants during the blossoming stage, when the vegetative processes become less active and the reproductive and seed-forming processes begin.

In some recent work by McCall (6), the active life period of the wheat plant was divided to cover three stages in its development: the first 30-day period, the second 30-day period, and finally the period extending from the close of the second 30 days to the maturity of the plant. McCall was able to show that the mineral requirements of the wheat plant during the first and the second 30-day periods were substantially the same, but the salt proportions producing high and low yields during the third growth period were markedly different from those giving corresponding yields during the first and second 30-day growth periods. While this does not directly apply to the salt requirements of the buckwheat plants here employed, it strongly suggests the possibility that the change in physiological requirements of these plants may take place during the flowering stage or during the period extending from the flowering stage to the maturity of the plants.

SUMMARY

The preceding pages present the results of a comparative study of the salt requirements of buckwheat plants during two different developmental periods. The plants were grown in sand cultures supplied with nutrient solutions of the same initial total osmotic concentration value of 1.75 atmospheres, but differing in the proportions of the component salts. The series of solutions supplied to the sand cultures comprised 36 different sets of salt proportions of the three salts KH_2PO_4, $Ca(NO_3)_2$, and $MgSO_4$.

The results obtained from the plants grown during the first 4 weeks after germination, in sand cultures supplied with nutrient solutions, were compared with those obtained from older plants grown during the period of development

between the flowering stage and maturity, in sand cultures supplied with the same solutions. The main facts brought out by this comparative study are briefly summarized as follows:

1. The highest yield of buckwheat tops obtained in a period of 4 weeks directly following germination occurred with the sand culture supplied with a solution having the following salt proportions: KH_2PO_4, 0.0144 m.; $Ca(NO_3)_2$, 0.0052 m.; and $MgSO_4$, 0.0200 m. The highest yield of tops, of roots, and of seeds was obtained, during the second developmental period, from a sand culture supplied with a solution having the salt proportions of KH_2PO_4, 0.0108 m.; $Ca(NO_3)_2$, 0.0130 m.; and $MgSO_4$, 0.0100 m. Thus the maximum yields were produced during the late developmental period in a sand culture furnished with a solution characterized by having a lower proportion of mono-potassium phosphate, a much higher proportion of calcium nitrate and a much lower one of magnesium sulfate than had the solution supplied to the sand culture giving the highest yield of tops during the early period of development.

2. The salt proportions of the solution in the sand culture of the present series producing the maximum yield of tops during the early developmental period, and those giving the highest yields of tops and of roots during the late period of development, are in exact agreement with those giving maximum yields of tops and of roots in the corresponding series of solution cultures previously carried out.

3. Under the conditions of these experiments the physiological properties of the nutrient solutions, as these affect the growth of the plants, are not altered to any marked extent when the solutions are added to the sand cultures.

4. High yields of tops and of roots are, in general, associated with relatively low values—but not the lowest values—of the three cation ratios Mg/Ca, Mg/K, and Ca/K. The values of these ratios characterizing the solutions supplied to the sand cultures, show pronounced differences for the cultures giving maximum yields of tops, and maximum yields of roots, and also for those giving minimum yields of roots, during the two different developmental periods of growth.

5. High transpirational water loss is associated with high yields of tops and of roots, and low transpiration with low yields

6. For each of the two developmental periods of growth, high water requirement is, in general, associated with low yields of tops and of roots, and low water requirement with high yields.

7. The relation of the growth rates of the buckwheat plants to the variations in the osmotic proportions of the solutions supplied to the sand culture is markedly different for the two different developmental periods of growth, whether this relation is judged by the criterion of tops or of roots, by that of transpiration, or by that of the water requirements of tops or of roots.

REFERENCES

(1) BRIGGS, L. J., AND SHANTZ, H. L. 1911 A wax seal method for determining the lower limit of available soil moisture. *In* Bot. Gaz., v. 51, p. 210–219.

(2) HILGARD, E. W. 1911 Soils, their Formation, Properties and Composition, p. 209. New York.

(3) LIVINGSTON, B. E. 1915 Atmometry and the porous cup atmometer. *In* Plant World, v. 18, p. 21–30, 51–74, 95–111, 143–149.

(4) MCCALL, A. G. 1915 A new method for the study of plant nutrients in sand cultures. *In* Jour. Amer. Soc. Agron., v. 7, no. 5, p. 250–252.

(5) MCCALL, A. G. 1916 The physiological balance of nutrient solutions for plants in sand cultures. *In* Soil Sci., v. 2, no. 3, p. 207–253.

(6) MCCALL, A. G. 1918 Mineral food requirements of the wheat plant at different stages of its development. *In* Jour. Amer. Soc. Agron., v. 10, no. 3, p. 127–134.

(7) SHIVE, J. W. 1915 A study of physiological balance in nutrient media. *In* Physiol. Res., v. 1, no. 7, p. 327–397.

(8) SHIVE, J. W., AND MARTIN, W. H. 1918 A comparison of salt requirements for young and for mature buckwheat plants in water and sand cultures. *In* Amer. Jour. Bot., v. 5, no. 4, p. 186–191.

(9) SHIVE, J. W., AND MARTIN, W. H. 1918 A comparative study of salt requirements for young and for mature buckwheat plants in solution cultures. *In* Jour Agr. Res., v. 14, no. 4, p. 151–175.

(10) TOTTINGHAM, W. E. 1914 A quantitative chemical and physiological study of nutrient solutions for plant cultures. *In* Physiol. Res., v. 1, no. 4, p. 133–245.

SOIL ACIDITY METHODS

R. E. STEPHENSON

Iowa State Agricultural College

Received for publication June 10, 1918

I. A COMPARISON OF SEVERAL METHODS

Introduction[1]

It is questionable whether any method now in use gives correctly the absolute acidity in the soil, and probably since there is such a wide variation in results, most of them do not even approximate it. But even though total acidity cannot be measured accurately, it is very desirable that reliable comparative results should be obtained. The first problem in the study of soil reaction, therefore, is the selection and standardization of a method. This same problem is met in many studies, but probably never more emphatically than in an investigation of the nature and significance of soil acids.

In this work, the study of methods has been undertaken only as preliminary to other investigations, the object being merely to select a method suitable for use. The methods taken up are typical of those now employed in soils work, and include the Hopkins, Veitch, Jones, MacIntire, Truog and Tacke methods. Many modifications of these more or less standard methods have been devised by various workers, but the consideration given here covers the principles involved in the operation of the methods in general.

Experimental procedure

Hopkins. A rather popular method is that put forward by Hopkins (2) of Illinois. Results here depend upon the liberation of acid from a neutral salt, or upon an exchange of bases freeing iron and aluminum from zeolites or other minerals, which action amounts to the same as that first mentioned, namely, a free acidity. The action is brought about by shaking the acid soil and a salt together for three hours. A solution of common salt was originally used. Later potassium nitrate was found more reactive and substituted for the salt. In this work sodium nitrate has been employed but the action is probably approximately the same.

[1] This opportunity is taken to extend acknowledgments to Dr. R. S. Potter for the use of his laboratory and for helpful consultations during the progress of the work; also to Dr. P. E. Brown for suggestions and criticisms offered at various times.

Jones. The Jones method (3) is similar in type. In this case calcium acetate is used, and the application is made dry, mixing primarily by trituration, though solution and more intimate contact occurs when water is added. The two methods, though based upon identical principles, give a wide difference in the lime requirement as shown by the tests.

MacIntire. The procedure (4) used in this method consists of treating the soil with N/28 calcium bicarbonate, and evaporating to a thin paste on a steam bath. During this process the bicarbonate unites with and neutralizes the soil acids.

Veitch. This method (9) makes use of N/28 calcium hydroxide to neutralize the soil acids, and in the treatment which indicates the lime requirement there is no excess of base present. In this work, to facilitate speed, the first evaporation was carried out on a hot plate until there was danger of spattering, finishing on a steam bath.

Truog. For this test (8) 4/10 normal barium hydroxide is used to neutralize the acidity. The acid soil and base are allowed to react for just one minute, during which time the active acidity is supposedly neutralized. The mixture is then evaporated to dryness on a water bath, before determining the excess of base employed.

Tacke. The Tacke method (6) makes use of pure calcium carbonate and water to bring about the neutralization of soil acids. The original technique was used except for such changes as are mentioned later in the discussion. No heat is employed, but intimate contact between the soil and lime is secured by vigorous and continuous shaking. As the reaction occurs, carbon dioxide is evolved and removed by aeration. The amount of carbon dioxide evolved determines the amount of acidity.

Discussion of results

In these studies two soils which are designated as brown silt loam and gray silt loam have been used with all the methods. The names given are not presumed to describe the soils accurately, but the distinction is sufficiently exact for practical work.

The summary table presents some interesting facts. For the sake of comparison the lime requirement indicated by the Tacke method is given the value 100, and the relative value is recorded for the other methods. The results are expressed in pounds of calcium carbonate per 2,000,000 pounds of soil.

It is observed that the Veitch method gives results which although rather inconsistent agree fairly well with those secured by the Tacke method and that none of the other methods agree very closely on either soil. This is in favor of the Tacke procedure, since the Veitch determination, even if not entirely reliable, should more nearly indicate the approximate neutral point than those methods where strong bases or a preponderance of carbonate is

used at high temperatures. In other works the modified Tacke method gives as nearly the true lime requirement and is much more consistent than any other test tried.

Another noticeable thing is that the methods do not vary in the same way with the different soils. This is indicative of a difference in reactivity of the acids of the two soils. It emphasizes, likewise, the unreliability of quantitative indications as well as the difficulties of accurate qualitative studies, where very different soils and treatments are employed. But a consideration of the nature of soil acidity leads to the prediction of such results.

True acidity has been defined as a hydrogen-ion concentration greater than that of pure water. In other words, regardless of how it is produced, there is no other acidity, in the soil or elsewhere, than hydrogen-ion. Hydrogen-ion concentration must be, therefore, the predominating factor in determining the injury which soil acids may cause to crops, either directly or indirectly,

TABLE 1

Table showing the lime requirement by the different methods on two soils

METHOD	LIME REQUIREMENT			
	Gray silt loam		Brown silt loam	
	Pounds per acre	Value	Pounds per acre	Value
Tacke (10 hr.)	4300	100.0	6500	100.0
Hopkins	2000	46.5	2400	36.9
Jones	3857	89.7	4821	74.2
MacIntire	3675	85.4	4070	62.6
Veitch	4648	108.1	6330	97.4
Truog	12200	283.7	15940	245.2

through their action upon soil microörganisms. Any poisoning which may be due to other specific ions is not here considered an acidity phenomenon. Recognizing this as the correct point of view, it becomes evident that there may be a high potential acidity without an injurious concentration of hydrogen-ion. As an example, a soil which contains sand has a potential acidity, represented by all the sand present. To how great an extent the silicon dioxide may be hydrolyzed to silicic acid in the soil cannot be determined, and to what extent this reaction may occur during the process of measuring the acidity by some of the common methods, it is likewise at present impossible to calculate. With confidence, it may be said that such reactions take place slowly under field conditions. In the field, too, reverse reactions must occur, in the dehydration of the silicic acid and the carbonating of calcium silicate. These reactions, likewise, must usually be slow.

But, nevertheless, in the soil even the salt calcium silicate represents also a potential acidity. If, when nitric acid is produced by the nitrifying organisms,

it reacts as it might with calcium silicate, soluble calcium nitrate would be produced, some of which would be removed by leaching, leaving relatively insoluble silicic acid. This reaction, too, must occur slowly and only to a limited extent under normal conditions. Though this reaction would furnish nothing but relatively inactive acid, which may not be directly harmful, the results are quite different when aluminum or iron salts are liberated. To what extent these reactions occur is also somewhat a matter of speculation though some investigations have been made to determine the iron and aluminum liberated by neutral salts. Since this work has been only a test of methods, other phases of the acidity problem have been investigated only in a very limited way, and little can be said in regard to the specific problems of acidity except as they have been brought out in the general study. It is very probable, however, that some of these potential acids affect the lime requirement as indicated by the different methods, and sufficient data are at hand to present some pertinent criticisms of the methods tested.

Objections to the methods

Hopkins vs. Jones. Since these two methods are so much alike they are discussed together. A wide difference between them is shown, the Jones method giving more than three times as high a lime requirement as the Hopkins. A different factor has been used at different times for the Hopkins method, the highest one which has been noted being 4. But even this factor ($2\frac{1}{2}$ was used in the calculations) does not make the requirement at all comparable with that indicated by the use of calcium acetate. It should not, however, be expected that the two methods would agree. Jones with his method uses no factor, assuming that the reaction gives the correct lime requirement. Since nitric and acetic acids do not have strengths of the same order of magnitude, equivalent amounts of each would not be set free by the soil acids. Then too, there is the difference in solubility of the respective salts of the soil acids with sodium and calcium. Since calcium salts, as a rule, are much less soluble, there will be a more complete displacement of the acid from calcium than from sodium salts. It is logical, therefore, that a higher result should be obtained with the calcium salt of a weak acid than with the sodium or potassium salt of a strong acid. That the Hopkins procedure would give different results with different salts used in making the extraction also is without question.

Finally, since the effective strength of acids depends upon solubility and ionization, and since soil acids may possess greatly varying effective strengths, it is not probable that soils with the same potential acidity would give at all comparable hydrogen-ion concentrations. The amount of acid liberated from a neutral salt therefore, would be quite variable with different soils for a single extraction, and the factor used should vary accordingly in order to adapt

the method to all soils. To secure a variable factor of this type that would operate accurately would be impossible. An acid soil which gives a high hydrogen-ion concentration would require fewer extractions to remove the total acidity and, therefore, a smaller factor. A very unreactive acid would be extracted only with difficulty, if at all. The data given here indicate, however, that a considerable part of the soil acids of normal soils is sufficiently active to be obtained with comparative ease. This must evidently vary with soils, and though the method is hardly sensitive enough for such work, results show that acidity curves diverge at first and then finally tend to come together, these curves representing the rate of evolution of carbon dioxide which is the index of reactivity of the soil acids.

The conclusion seems justified, therefore, that neither of the above procedures can give results which are reliable and consistent when different soils and treatments are studied. But it is possible that many methods may be sufficiently accurate to indicate the amount of lime to apply to farm land. The farmer does not usually apply small fractions of tons and the distribution

TABLE 2

Lime requirement in pounds of $CaCO_3$ *per 2,000,000 pounds of soil by the MacIntire method with varying amounts of soil*

SOIL	LIME REQUIREMENT		
	20 gm.	10 gm.	5 gm.
Brown silt loam	5,175	8,350	10,700
Soil blank	450	300	150

and mixing with the soil cannot be at all uniform. The recommendations made to him, too, are as a rule in excess of the indicated requirement when it is low, but below it when it is high. That a rough method which might serve this purpose would not be satisfactory for research work is quite evident.

MacIntire. The determinations by this method do not agree with the two previous results, though when the original directions were followed (except that a 20-gm. sample was used) they were rather close to those given by Jones' method. However, when tests were made with varying amounts of soil, a soil blank being used, the results given in table 2 were obtained.

The same amount of bicarbonate is used in each case, the amount of soil being the only variable. It is noted that the lime requirement depends very much upon the amount of soil used. When there is a large excess of bicarbonate, nearly double the amount is taken up, as a result of the influence of mass action. Hydrolytic reactions may exert a greater effect also. Since calcium hydroxide is soluble to the extent of one part in six hundred of water, more soluble than the carbonate or bicarbonate, and is a strong base relative to its concentration, hydrolysis would increase the action of the carbonate

upon the soil. These variations are sufficient objection to throw the method into disrepute. Ames and Schollenberger (1) in their work on different methods show that this method is very sensitive to variations in manipulation. The directions call for evaporation to a thin paste. This is a very indefinite end point, and other workers have found that both the degree of dryness and the rate at which it is evaporated cause a wide variation in the lime requirement. The same result is observed in this work in the two determinations. The increase in lime requirement with length of time of contact between moist soil and bicarbonate would indicate that an equilibrium is reached rather slowly. A large excess of lime with a small sample of soil would permit the attainment of equilibrium sooner and would be one cause of the higher requirement. The amount of organic matter decomposed also is greater when a longer time is allowed before the mixture of acid soil and lime is brought to complete dryness.

TABLE 3

Determinations of lime requirement in replication by the Veitch method, expressed as tons of $CaCO_3$ *per 2,000,000 pounds of soil*

NO.	LIME REQUIREMENT	
	Gray silt loam	Brown silt loam
	tons	*tons*
1	2.5	3.57
2	2.14	3.21
3		3.03
4		2.85

Veitch. Several tests were made by this method in an attempt to duplicate the determinations. Often not only did the results disagree, but either all soil treatments were alkaline or none were alkaline, and the test was apparently entirely outside the range of acidity. Never less than two treatments on each side of the previous approximately neutral indication were used. This gives a range of 1785 pounds, but many times it proved inadequate. Some characteristic variations are given.

As to the causes for the variations, there may be several. Some action, such as hydrolysis and the effect of organic matter and soil particles upon the indicator, have already been suggested by other workers (1). The rate at which the evaporation is carried out on the hot plate is a source of marked variations in the results as found in this work. When taken down rapidly (about 30 minutes on the hot plate) it is impossible to read the end point accurately. The rapid heating seems to deflocculate the soil colloids and the minute particles never settle out. When taken down slowly (about 1½ hours on the hot plate) this trouble does not occur, and a nearly clear liquid for the final reading may be obtained. It should be understood that the evaporation

is finished on a steam bath and there is no cooking of the soil from excessive heat.

The rate of evaporation on the water bath, however, seems to have less effect. But this rate cannot be augmented much since a temperature higher than that of steam cannot be obtained. Readings from the final evaporation are sometimes very difficult even when the liquid is quite clear. At the Ohio Station (1) filtering was resorted to in order to remove soil particles. These many variations in procedure must give decidedly different results. And what is worse, perhaps, it seems impossible to standardize the operations. As an example of lack of uniformity it may be pointed out that the final evaporation is a very indefinite process. The reading depends considerably upon the degree of dryness obtained and it is impossible to go always to the same point. Directions call for taking down to about 15 cc. in a 100-cc. beaker. The best guessing would probably vary at least 50 per cent from this amount, with a somewhat proportionate effect upon the reading. And too, there are the many ill effects which may be attributed to the application of heat to the soil during the determination.

After considerable work, double distilled neutral water being used part of the time to remove all source of error due to impure water, it was concluded that not only can one worker not duplicate the results of another by this method, but that the same worker may not obtain concordant results except as an accident. The method, therefore, cannot be considered suitable for accurate experimental work. The presence of large amounts of calcium carbonate which has been added to the soil in some of the work would also probably interfere with the successful use of the method.

Truog. The requirements indicated by this method are much higher than those given by any of the other determinations. It has been shown in another paper that this method is susceptible to an effect from varying the relative amounts of soil and alkali and from the dilution used when adding the alkali to the soil. Also, different bases give different results. The procedure seems, therefore, unreliable and gives an unreasonably high lime requirement partly because of mass-action effects. Since one of the products of the reaction of the base with the soil acids is water, which has the same effect as removing one of the products of the reaction, the equilibrium must be pushed far in that direction, and also the strong alkali may decompose organic material in the soil, and thereby indicate an unduly high acidity. Normally the decomposition of organic materials may occur slowly enough that most of the acids produced are oxidized as rapidly as they are liberated. Mineral acids are apparently the only source of a stable soil acidity. In experiments by Temple (7) where organic acids are added to the soil they were found to disappear completely in two weeks. Bases held as organic salts are, therefore, likely to be freed to act again in neutralizing the more permanent acids. Partly, for this reason, soils high in organic matter are less harmfully affected by a given degree of acidity.

Conclusion

After a preliminary test of the various methods, the results seem to indicate that one method only is reliable for research work. This is a modification of the old Tacke procedure quite popular in Europe, but as yet little used in this country. Accordingly, this method was taken up in an attempt to develop it to a higher degree of efficiency.

II. TACKE METHOD

The general action of methods

There must always be severe criticism to offer for any method of determining soil acidity where a strong base or heat is employed in the determination. Heat increases hydrolytic reactions and augments errors of this source. Heat, too, decomposes organic matter, as do also strong bases, therefore, indicating an unduly high and often inconsistent lime requirement. A modification of the Tacke procedure which originated at the Ohio Station (1)

TABLE 4

Lime requirement by the Tacke method, comparing pure water and saturated $CaCl_2$, *and varying the time of running*

TREATMENT	LIME REQUIREMENT			
	3 hrs.	6 hrs.	9 hrs.	21 hrs.
	lbs.	*lbs.*	*lbs.*	*lbs.*
100 cc. pure H_2O and 2 gm. $CaCO_3$..	5,000	6,100	6,500	7,000
50 cc. saturated $CaCl_2$ and 2 gm. $CaCO_3$.....	3,900	5,150		5,475

and which we have not tried, makes use of heat in a partial vacuum to hasten the reaction. In this manner a temperature no higher than 50°C. may be maintained, thereby reducing to a minimum the decomposition of organic matter by heat. Nevertheless, the authors report a high lime requirement, and it has seemed advisable to lengthen the time of contact between soil and carbonate rather than attempt to gain speed by the use of heat.

The end point of the various reactions occurring with the different methods used depends very much upon solubilities. Three type reactions are given:

$$NaOH + HX \rightleftarrows NaX + HOH \quad (1)$$
$$NaCl + HX \rightleftarrows NaX + HCl \quad (2)$$
$$Ca(HCO_3)_2 + 2HX \rightleftarrows CaX_2 + 2H_2CO_3 \quad (3)$$

Here HX may represent any soil acid. It is not necessary that the X represent any particular valency except for the sake of giving balanced equations. In fact X might as well stand for a very complex acid radical. The principle involved remains the same. It is quite evident that in reactions such as shown

in equation (1), where water is one product, the equilibrium is far removed to the right. The reaction occurs rapidly because of the solubility and strength of the base used. A very insoluble salt would produce the same effect on the equilibrium as the water. It is just as evident that reactions similar to that shown in equation (2) can not go far to the right. Solubility and ionization of the reaction products on either side of the equation must be considered in the equilibrium systems. Of course soil reactions are not so simple as this but the results are illustrative of the principles involved. In equation (3) is shown the reaction which must occur with the use of the Tacke method. The removal of the carbon dioxide must carry this equilibrium far to the right according to the mass law. The reaction proceeds slowly, however, because of the slight solubility of both the base and the soil acids.

Perhaps there is no strong argument for using the same materials in a determination of soil acidity as are used in correcting this condition in the field. In fact, there are many bases in the soil which have a neutralizing power. Yet, there is no proof that finely-divided calcium carbonate brought nto intimate contact with the acid soil by vigorous shaking, mixing, and aerating, should not give the true lime requirement. The results of this method with the various modifications tested are presented below.

Procedure

The method (6) was designed originally for European moor soils, high in organic matter. The soils used here, however, are mineral, but the method has proved no less satisfactory with them. Previously in this country the procedure has been tried to some extent by Wheeler, Hartwell, and Sargent (10). No one, however, has ever made use of the vigorous shaking which is emphasized here.

The procedure consists in treating the soil directly with calcium carbonate in 500-cc. Kjeldahl flasks, using a 20-gm. sample of soil and about 2 gm. of carbonate, with 50 cc. of carbon-dioxide-free water. The carbonate is introduced as a water suspension. The limestone reacts with the soil acids and the carbon dioxide thus liberated is collected in sodium hydroxide towers. The absorption towers are cylinders holding about 150 cc. and the sodium hydroxide, of which 100 cc. is used, has a strength of approximately 2.5 per cent. Two rubber discs in the cylinders and traps on top aid in the absorption of the carbon dioxide. The machine provides for 10 Kjeldahl flasks and likewise, 10 towers are needed. In all of these tests where carbon dioxide is collected the Kjeldahl flasks are swept out with air purified by passing through soda lime before starting the determination. Originally hydrogen was used to secure aeration, but in this work purified air has proved more convenient and perfectly satisfactory.

During the process, as suggested above, continuous and vigorous shaking is provided. The power is secured by attaching to an electric fan motor.

That thorough mixing is insured is evident from the fact that the flasks travel a path of 2 inches, with a back and forth somewhat rotary movement, about 400 times each minute. This gives 240,000 shakes in the 10-hour run. These figures are given merely to emphasize the difference between the modifica ion presented here and an occasional shaking by hand. The 10-hour run is adopted partly for convenience and partly for the sake of accuracy.

With this method, as with the Truog and MacIntire methods, double titration with phenolphthalein and methyl orange is necessary. There is always a source of error in such a titration, but its magnitude seems insignificant in comparison with other variations. When a 20-gm. soil sample is used and the carbon dioxide titrated against N/10 acid, each cubic centimeter of the titration represents a lime requirement of 1000 pounds of calcium carbonate per 2,000,000 pounds of soil. In the results, a blank on both the soil and the alkali used for absorption is always subtracted. More accurate titrations can be made when the amount of carbon dioxide is not excessive. The end point is not distinct when the carbon dioxide evolved is the equivalent of more than about 20 cc. of N/10 acid. The amount of carbon dioxide may, of course, be regulated somewhat by diminishing the size of the soil sample in case there is too much for accurate titration.

Modifications

In trying to improve the method a number of variations have been used. The solubility of calcium carbonate in water at 20° C. is about 1.2 parts per 100,000. In N/10 sodium chloride it is three times as great. If the solubility of the carbonate were the limiting factor in the reaction, then the use of a dilute sodium chloride solution instead of pure water should hasten the evolution of carbon dioxide. The carbon dioxide is less soluble in the presence of chlorides and, therefore, aeration should be facilitated also. The test, however, shows little effect on the final results. While a strong solution of chloride, has a depressing effect upon the solubility of lime, it would prevent any bacterial action which might liberate carbon dioxide. Süchting, in an attempt to improve the method, has suggested that there is a fermentative action which occurs, introducing an error in the determination, and it was thought at one time that this might be a factor to consider. But since a soil blank is run, such a provision seems superfluous. The blank on the soil is always small, seldom equivalent to more than 1 cc. of N/10 acid. It is also more or less constant in value for the different soils, and considering the results where antiseptic conditions have been provided it may be concluded that with a blank, the method is perfectly reliable, without any provision to reduce fermentations which might occur.

Calcium chloride, which was used in a similar way, has both the effect of increasing the solubility of lime with an accompanying facility of aeration, and

the common ion effect. Still another modification was the use of a few drops of toluene in the water. This should insure sterilization when long runs are being made and it seems also to have an effect of speeding up the reaction. The stimulation may be due to some chemical change brought about with the soil as has been suggested to occur in partial sterilization studies made by Pickering (5). Should any chemical change occur it would likely become a source of error rather than an advantage to the method.

A modification which should theoretically prove effective in hastening the reaction is the use of sodium nitrate solution. The sodium nitrate should liberate the soil acids in the same way as in the Hopkins method and the calcium carbonate which is present in large excess, should neutralize the acids as fast as liberated. The effect should be to permit the reaction to go to completion as though a number of extractions were made according to the original Hopkins plan. In actual practice the reaction is hastened, but it has not yet been considered of special value. The recovery should apparently be similar also, when chlorides of calcium and sodium are used, but the action has sometimes proved depressing rather than stimulating. These salts have a precipitating effect upon the soil colloids, as is observed by the clear supernatant liquid a short time after the machine has stopped shaking. An action of this kind might account for the retardation of the reaction, perhaps by rendering the soil acids more slowly soluble, and a consequent lower lime requirement is indicated. But the vigorous shaking has the reverse effect of breaking up the soil and apparently increasing the amount of colloidal material. It is only on very long runs that there is a marked indication of this kind, however.

The predominating factors with the method so far as studied seem to be the thoroughness of shaking and length of time of running. It has been found that scarcely any reaction occurs in several hours when the mixtures stand without shaking. It is found also that the evolution of carbon dioxide is a continuous process and probably never entirely ceases. In time, however, evolution is so slow that the amount evolved is little more than that ascribable to experimental error. This might seem to throw the method into disrepute, but these studies have led to a high valuation of the method. Below are given in more detail some comparisons and results.

Water vs. saturated calcium chloride[2]

In this determination 100 cc. of water was used, but a smaller amount has since been decided upon as adequate and more expeditious to the aeration.

The results in table 4 show the depressing effect of a concentrated calcium chloride solution upon the rate at which carbon dioxide is evolved. Though

[2] All results unless otherwise stated are expressed as pounds of $CaCO_3$ per 2,000,000 pounds of soil.

the carbon dioxide is less soluble in concentrated chloride solution and should, therefore, aerate more rapidly than from pure water, the reaction is markedly retarded, probably on account of the insolubility of the carbonate in the concentrated calcium chloride. Evidently, too, the hydrochloric acid is not replaced to any extent from the calcium chloride by the soil acids, or else the reaction would have been speeded up. It is easily observed, also, that the evolution of carbon dioxide gradually decreases in rate, by far the greater part having come off during the first three hours.

Dilute solutions of sodium and calcium chlorides

There is little difference in the effect of dilute solutions of the chlorides of sodium and calcium, as shown in table 5.

TABLE 5

Lime requirement by the Tacke method, comparing 5 per cent NaCl *and 5 per cent* $CaCl_2$, *and varying the time of running*

TREATMENT	LIME REQUIREMENT		
	5 hrs.	8 hrs.	21 hrs.
	lbs.	*lbs.*	*lbs.*
50 cc. 5 per cent NaCl and 2 gm. $CaCO_3$.....	5,725	6,750	7,050
50 cc. 5 per cent $CaCl_2$ and 2 gm. $CaCO_3$.....	5,660	6,480	7,020

Probably the dilute salts hasten the reaction somewhat but the effect is not marked, as is shown by comparing the requirement indicated here at the end of 8 hours with that shown at the end of 9 hours when pure water is used. These figures indicate again, as do all others, that the evolution of carbon dioxide has slowed up very much by the end of 8 hours.

The effect of varying the amount of soil and carbonate used

To determine the effect of varying the amount of soil, 50 gm. were used in comparison with a 20-gm. sample.

TABLE 6

Lime requirement by the Tacke method, showing effect of varying the amount of soil used, with two periods of running

AMOUNT OF SOIL	TREATMENT	LIME REQUIREMENT	
		9 hrs.	21 hrs.
gm.		*lbs.*	*lbs.*
20	50 cc. pure H_2O and 2 gm. $CaCO_3$.....	5,925	7,350
50	50 cc. pure H_2O and 2 gm. $CaCO_3$.....	5,790	7,300

The results are evidently not affected by the size of the soil sample so long as it is not too large to prevent intimate mixing with the carbonate and aeration is not depressed. It will be observed that there is some variation in corresponding runs from day to day. This is due partly to variations in sampling the soil, to humidity conditions and other factors which cannot be controlled. An air-dry soil must take up considerably more water in a saturated atmosphere than in one that is relatively dry, and consequently not the same actual weight of soil is used in all tests. These errors, however, are not significant for the purposes of the tests made and the method is no more susceptible than others in these respects.

The amount of carbonate also was varied, 5 gm. being used instead of 2 gm., and this proved likewise to be without influence on the determination.

In this test 10 per cent calcium chloride solutions were used, but comparative results could not have been different with pure water. The only essential, therefore, is that there shall be an excess of carbonate and sufficient water to insure intimate mixing. A large amount of water retards aeration very decidedly, as was found in standardizing a bicarbonate solution by this method of decomposing carbonates.

TABLE 7

Lime requirement by the Tacke method showing the effect of varying the amount of $CaCO_3$ *used in a dilute* $CaCl_2$ *solution*

TREATMENT	LIME REQUIREMENT
	lbs.
50 cc. 10 per cent $CaCl_2$ and 2 gm. $CaCO_3$	6,825
50 cc. 10 per cent $CaCl_2$ and 5 gm. $CaCO_3$	6,700

Period of running, 9 hours. Amount of soil used, 20 gm.

Incidentally, we may observe here the speeding-up of effect a dilute solution of the chloride of calcium. When this result is compared with the requirement indicated by pure water at the end of 9 hours, a difference of about 1000 pounds per acre is observed. Any method becomes more consistent, however, with familiarity and standardization, and it is quite probable that some of the variations could be reduced if all operations were carried out under a rigidly-controlled procedure. Therefore, it is not desirable to over-emphasize apparent effects of different treatments.

Effect of toluene

It should be understood that only a few cubic centimeters of toluene were added to pure water in this test, usually about 2 cc. This is too small an amount to have any marked effect upon the solubility of either the carbonate or the carbon dioxide evolved. The results are given in table 8.

TABLE 8

Lime requirement by the Tacke method, showing the effect of toluene compared with pure water and $CaCl_2$ *solution*

TREATMENT	LIME REQUIREMENT		
	9 hrs.	24 hrs.	36 hrs.
	lbs.	*lbs.*	*lbs.*
50 cc. H_2O, 2 gm. $CaCO_3$ and toluene	6,600	7,200	8,400
50 cc. $CaCl_2$ and 2 gm. $CaCO_3$		7,175	8,200
50 cc. pure H_2O and 2 gm. $CaCO_3$	6,200		

The toluene seems to have somewhat stimulating action on the rate of evolution of carbon dioxide during the first 9 hours. Where the vacancies are shown, the determinations were not made. In general, it may be said that none of the compounds tried have been of value to the procedure, and their use for routine work has been rejected.

Results with gray silt loam

A few determinations were made with the second soil which shows the same general behavior. In laboratory work the method has been used with still other soils and so far as tested, it works equally well with all.

TABLE 9

Lime requirement by the Tacke method showing the effect of toluene compared with water and $CaCl_2$ *on the gray silt loam soil*

TREATMENT	LIME REQUIREMENT		
	9 hrs.	24 hrs.	36 hrs.
	lbs.	*lbs.*	*lbs.*
50 cc. H_2O, 2 gm. $CaCO_3$ and toluene	4,400	5,800	
50 cc. 10 per cent $CaCl_2$ and 2 gm. $CaCO_3$	4,200	5,600	
50 cc. $CaCl_2$ and 2 gm. $CaCO_3$	4,425	5,700	6,700

The only reason for using toluene in the water with this soil is that the run happened to be made when the effects of toluene were being tried in comparison with calcium chloride solutions. The water alone could not have acted much differently. It is observed that there is considerable carbon dioxide evolved after the first 9 hours' run. This would indicate that either the aeration was too slow to permit the reaction to go forward rapidly, or that the acids of the soil were inactive. Both factors may have operated in this case, and the results emphasize the importance of maintaining standard and control conditions to allow the reaction to occur at the maximum rate.

Discussion of factors influencing results

Temperature. There are possible factors influencing the operation of the method, for which no accurate means of measuring the effect is available. Temperature increase means an increase in the rate of most chemical reactions, but it also means a decrease in the solubility of the carbonate, and there is no means of measuring the net effect of such variations. Even in the laboratory, if winter and summer extremes are compared, there must be large temperature ranges. This is one argument for continuing the shaking several hours. The long run tends to overcome momentary effects of temperature and other fluctuations.

Rate of aeration. A factor which is more important is the rate at which air is drawn through to carry over the evolved carbon dioxide. Though the stream of air may be regulated by means of glass stop-cocks of approximately the same bore and the size and rate of bubbles produced in the aeration serve as a guide, nevertheless, there is no quantitative check on the process. In general a rapid aeration has proved best, and a fairly rapid bubbling is perfectly safe from loss of carbon dioxide. In trials made, carbon dioxide has never been carried over into a second absorption tower. With a very large quantity of carbon dioxide, as might occur when residual carbonates are determined with this apparatus, there might be trouble and under such conditions it would be advisable to aerate slowly at first to insure complete absorption, and then after the first large evolution of gas has passed, to aerate more rapidly. At the end of the run it is especially advisable to speed up to secure as nearly complete aeration of the carbon dioxide as possible.

Pressure of carbon dioxide. Another factor which may influence the rate of reaction is the partial pressure of carbon dioxide in the Kjeldahl flasks where the reaction is occurring. Calcium bicarbonate is more than thirty times as soluble in water which is saturated with carbon dioxide as it is in pure water. Though saturation would never exist, the amount of carbon dioxide present at times is doubtless great enough to produce some effect. An excess of carbon dioxide would operate in another way, tending to retard the reaction according to the mass law. Here again the net effect is doubtful.

Nature of soil acids. Still another very important factor is the kind and strength of the acids occurring in the soil. The rate of reaction of calcium carbonate with soils must vary in proportion to the activity of the acids present, as previously suggested. It is quite evident that if this is true, the contact of soil and carbonate must be continued until the inequality in rate of reaction is overcome by the long-time run, or until there is a somewhat constant evolution of carbon dioxide in both cases. If run a short time only, one soil should show a much greater acidity than the other, while at the end of a sufficient time there should be little difference. For these reasons, it is believed that an extended time of shaking and aeration, tends to minimize a number of sources of error.

The rate at which carbon dioxide is evolved may be partially accounted for if the soil itself, not its solution, is highly "buffered," as would be manifested in a marked tendency of the soil mass to resist change in reaction with the addition of a base. To show buffer action it is necessary only that there be present a weak acid and the salt of the weak acid, or in the case of alkalinity a base and salt of similar nature. Without question many soils present ideal conditions for such a buffer action. This is indicated also by the fact that it is seldom possible to obtain soil acids in a water extraction. Insolubility alone would, of course, account for this phenomenon were it not for the fact that some soluble acids must be present and would be extracted were they not buffered. A logical way of accounting for their inactivity is found in this explanation. A buffer effect may account, therefore, for the large reserve acidity shown by some soils. Though the methods may indicate a large lime requirement the hydrogen-ion concentration is small. This may explain also the activity of microörganisms in very acid soils. The acidity reported is mostly reserve acidity and the organisms, for example the nitrifiers, are

TABLE 10

Lime requirements by the Tacke method, showing a comparison of the effect of normal nitrate solution with that of pure water

TREATMENT	LIME REQUIREMENT	
	3 hrs.	10 hrs.
	lbs.	*lbs.*
50 cc. pure H_2O, and 2 gm. $CaCO_3$	6,000	6,600
50 cc. N/1 $NaNO_3$ and 2 gm. $CaCO_3$	6,800	

active because the harmful acidity or hydrogen-ion concentration is below that concentration which is inimical to their life processes. Though the Tacke procedure does not measure the hydrogen-ion concentration accurately, it does measure the rate of reaction between carbonate and soil acids and, therefore, indicates the degree of activity and probable harmfulness of the acidity. This, rather than total acidity, is really the important point in a determination, and unless a method indicates something in regard to the activity of the acids it is not of the greatest practical value.

That much of the soil acidity is potential rather than active is indicated by some further results. Hopkins believes as suggested above that repeated extractions of the soil, taking the sum of all the fractions thus obtained, will give the total acidity. The data given in table 10 with normal sodium nitrate are notable.

In the 10-hour run with pure water the soil used shows an acidity of 6600 pounds, or 200 pounds less than the amount obtained in the 3-hour run with sodium nitrate. Since this investigation has been only started, it is not yet

possible to draw any conclusions. But it should be expected that many of the soil acids would be too weak to displace a strong acid, such as nitric, from its salt. However, since calcium carbonate is present to neutralize the acid as fast as liberated and since, too, the carbonate itself reacts directly with the inactive soil acids, the use of sodium nitrate might facilitate the activity of what are sometimes called "latent" acids. The nitrate increases the solubility of the carbonate very decidedly also, since calcium nitrate is soluble to the extent of one part in two of water. Any effect of this kind must tend to hasten the reaction.

More evidence is found in the test recorded in table 11, that carbon dioxide practically never ceases to be evolved.

It is easily observed that the rate of evolution of gas gradually decreases, and it seems probable that it should have finally a more or less constant value. The proper place to stop becomes, therefore, the important question. With former workers, the constant evolution of carbon dioxide has been the big objection to the method. Nevertheless, there are some good reasons for confidence in the method. The constant evolution of carbon dioxide means only that the same thing is occurring as takes place in the field. Namely,

TABLE 11

Lime requirement by the Tacke method, showing continuous evolution of CO_2

TREATMENT	LIME REQUIREMENT						
	9 hrs.	24 hrs.	36 hrs.	48 hrs.	72 hrs.	96 hrs.	120 hrs.
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
50 cc. $CaCl_2$ and 2 gm. $CaCO_3$....	6,700	7,175	8,200	8,950	9,275	9,575	10,075

that as fast as the acids present are neutralized, more develop, the rate of development being perhaps infinitely slow normally, but under the conditions of the test speeded up to a measurable rate.

This is doubtless partly due to buffering effects. In the field, too, there is the leaching of bases and the assimilation of bases by plants. Probably however, most plants use nearly as much of the acid radical. At least the production of crops must tend toward the preservation of bases, since the decay of plant roots and other residues leaves the basic radical in the soil, while the organic acid radical is completely oxidized under normal conditions. And when a base is leached there is removed with it an equivalent amount of acid in the form of its salt. Only, therefore, when a bicarbonate is leached is a functioning base removed, and increase in active soil acidity is not primarily the result of leaching of bases but rather the result of the development of new acids in the absence of base. So long as a base is in combination with an active acid it is useless for further neutralizing action. The continual leaching of salts, however, must finally result in a base-poor soil and lack of basic elements

for plant growth. Then when new acids develop, plant growth is still further retarded until the acidity is neutralized by application of lime. But the Tacke method is no less accurate in measuring the existing acidity whatever the rate or manner of its accumulation in the soil.

It is found, in unpublished results, that where lime is applied in large excess of the acidity, the method always indicates a lime requirement. This is apparently a correct indication. In the soil under field or greenhouse conditions, only the rather active acidity is neutralized because the base is not in sufficiently intimate contact with the soil. It is fairly certain that acid soils have local neutral or alkaline areas. It is just as logical that there must be local acid areas in any soil even though limed. In the operation of this method in the laboratory, however, with the vigorous shaking and constant aeration, more complete neutralization is secured. This, with the hydrolytic reactions that doubtless occur, gives a greater evolution of carbon dioxide than is actually represented by harmful acidity. In the field several years may be required for a similar action. It is known, however, that when soils are limed to the supposedly neutral point, they sometimes become acid again very quickly. Some experiment stations, therefore, always recommend an application of from one to three tons in excess of the indicated lime requirement. When methods differ so widely in their indications, lime requirement really means little unless both the method and type of soil are designated.

In this work some further data have been secured illustrative of the indefiniteness of the end point in the reaction between lime and acid soil. For a qualitative indication, the method originated by Truog has been used. This procedure, sometimes spoken of as the lead acetate method, has been investigated at the Ohio Station (1) and found as reliable as any qualitative test, so that it seems safe to trust its indication of the neutral point. The neutral point with the lead acetate test corresponds nearly exactly with the point where a constant acidity is shown by the Tacke method, running 10 hours. This is on acid soils (results unpublished) which have been limed at rates increasing successively by one ton, so that the soil acid equivalent in lime must occur somewhere in the series. It is evident, therefore, that the method goes beyond the point at which hydrogen-ions are liberated in sufficient quantity to free hydrogen sulfide from zinc sulfide, to an extent such that lead acetate paper is darkened. This excess or constant acidity is somewhere about 10 to 15 hundred pounds per acre, depending, of course, somewhat upon the length of time since liming the soil. These results indicate that a run for a shorter time on the soil used would be adequate. Possibly half as long might be sufficient. But the constant or excess requirement introduces no error in the studies, since its value is known and the excess is the same for all treatments within experimental error. To run a little in excess of the true lime requirement is only working on the side of safety. It might be argued, too, that this potential acidity should be provided for, since it may eventually

develop to a harmful extent in the field. For experiment station use, however, a run of from 5 to 7 hours would seem to give accurate and comparable results, the exact time depending upon the soil and somewhat upon convenience. The time of running is evidently largely susceptible to adaptation to routine work, and this without introducing any serious error.

Conclusions

The possibilities of the Tacke method are very encouraging though there are some weak points. Since there is only pure water, pure calcium carbonate and soil, with the accompanying reaction products, present in the equilibrium systems, the results should not be extreme in either deficit or excess, and probably they may be about correct quantitatively as well as accurate qualitatively.

If time permits it is hoped that further developments may be made to determine more exactly, if possible, the end point to consider in the reaction. It may be possible by the use of colored indicators and known hydrogen-ion concentration to follow the reaction in the decomposition of pure carbonate. By comparing this reaction rate with that of the soil, a more definite conclusion may be derived in regard to the length of time for conducting the test. Such studies should also give further knowledge in regard to the nature of soil acids, their activity, degree of buffering and other disputed points.

SUMMARY

Part I

1. Methods which depend upon the liberation of an acid from its salt do not give total acidity and indicate a lime requirement which depends both upon the soil and salt used.

2. Methods employing heat or a strong base are not reliable since their indications are likely to be both excessive and inconsistent.

3. The nature of the soil acids is a very important factor which must be considered in studies of lime requirement.

4. The Tacke method has proved the most satisfactory of any method tried.

Part II

From a study of the Tacke method of determining the lime requirement of soils, the following conclusions are indicated:

1. Pure water is a reliable medium for bringing about the reaction between the acid soil and carbonate.

2. The use of dilute solutions of calcium or sodium chloride hastens the reaction only to a limited extent.

3. A concentrated solution of the above salts may prevent fermentative reactions, but such a provision has proved unnecessary. The rate of reaction is somewhat depressed by concentrated chlorides.

4. Toluene has proved of no value to the method. An antiseptic is evidently not needed.

5. The use of normal sodium nitrate hastens the reaction but its value is not yet established.

6. The time of running, the rate of aeration and the vigor of shaking are the most important factors in the Tacke method.

7. The rate of aeration should be maintained at a maximum.

8. The effects of temperature and the partial pressure of carbon dioxide cannot be determined.

9. A long run, 5 to 10 hours, adds to the reliability of the method, tending to overcome many momentary influences.

10. The activity of soil acids varies greatly as measured by the rate of evolution of carbon dioxide. The more reactive acids react at once, the less reactive only after long contact and thorough mixing of soil and carbonate, and more complete removal of carbon dioxide liberated.

11. The method is not only consistent in indicating total acidity but in a limited way measures the toxicity of the soil acids.

REFERENCES

(1) AMES, J. W., AND SCHOLLENBERGER, C. J. 1916 Liming and lime requirement of soil. Ohio Agr. Exp. Sta. Bul. 306.

(2) HOPKINS, C. G., AND PETIT, J. H. 1910 Soil Fertility Laboratory Manual. New York.

(3) JONES, C. H. 1913 Method of determining the lime requirement of soils. *In* Amer. Fert., v. 39, no. 11, p. 28–29. *Abs. in* Chem. Abs., v. 9 (1915), no. 14, p. 1961.

(4) MACINTIRE, W. H. 1915 A method for the determination of the immediate lime requirement of soils. *In* Jour. Indus. Engin. Chem., v. 7, p. 227, 864.

(5) PICKERING, S. U. 1908 The action of heat and antiseptics on soils. *In* Jour. Agr. Sci., v. 3, p. 32.

(6) TACKE, B. 1897 The determination of free humus acids in moor soils. *In* Chem., Ztg., Bd. 21, No. 20, p. 174–175. Described by Wiley, H. W., Principles and Practices of Agricultural Analysis. 1906 Ed., v. 1, p. 373.

(7) TEMPLE, J. C. 1914 Nitrification in acid or non-basic soils. Ga. Agr. Exp. Sta. Bul. 103.

(8) TRUOG, E. 1915 A new apparatus for the determination of soil carbonates and new methods for the determination of soil acidity. *In* Jour. Indus. Engin. Chem., v. 8, no. 4, p. 341–345.

(9) VEITCH, F. P. 1902 The estimation of soil acidity and requirement of soils. *In* Jour. Amer. Chem. Soc., v. 24, p. 1120.

(10) WHEELER, H. J., HARTWELL, B. L., AND SARGENT, C. L. 1900 Chemical methods for ascertaining the lime requirement of soils. *In* Jour. Amer. Chem. Soc., v. 22, p. 153.

REPORT ON THE EXAMINATION OF COMMERCIAL CULTURES OF LEGUME-INFECTING BACTERIA

C. R. FELLERS

New Jersey Agricultural Experiment Station

Received for publication November 1, 1917

The object of this work was to test the efficiency, purity and identity of commercial cultures of nitrogen-gathering bacteria. Since the time of Caron (2) who first proposed inoculation of the soil with beneficial non-symbiotic nitrogen-gathering bacteria, and of Nobbe and Hiltner (4) who first used the method of seed inoculation, a great many cultures have been placed upon the market. Repeated failures with non-symbiotic nitrogen-gathering bacteria have led to a very limited use of such cultures at the present time. However, one firm's product (Alphano Humus Co.), which has been tested repeatedly by the author contains vast numbers of *Azotobacter*.

Greater by far is the importance of the symbiotic nitrogen-gatherers. With the discovery of the functions of nodules on the roots of legumes by Hellriegel (6) in 1886, a new epoch was begun in the science of agriculture. His announcement that the organisms within the nodules are able to assimilate elemental gaseous nitrogen from the atmosphere, has been verified so many times by scientists of high repute that no doubt exists today on this question.

Immediately after this discovery, men began to commercialize these nitrogen-gathering bacteria. Thus we find the work of Salfeld (8) and Schmitter (9) in Germany demonstrating the value of inoculating moor soils with soils infected with the nitrogen-gathering organisms, especially for the growth of legumes. Later on "Nitragin," a German product, appeared on the market. Contradictory results were obtained from its use, but the commercialization of the nitrogen-gathering bacteria gradually became well established and other preparations were manufactured and sold to farmers for the purpose of inoculating legumes. Harrison and Barlow, of the Ontario Agricultural College, did pioneer work in North America and originated the method of growing *Bacillus radicicola* on a nitrogen-free medium. This was soon copied by others until, at the present time, most of the cultures on the market are pure cultures of definite varieties of the nitrogen-gathering bacillus, grown on a nitrogen-free or, rather, a nitrogen-poor agar medium.

Recently, however, more attention has been paid to the distribution of *B. radicicola* in nature's own medium, i.e., the soil. Muck which has been specially treated is also being used to some extent. Liquid cultures have appeared

to be shorter-lived than the cultures on solid media, and hence their use is somewhat limited at the present time. The United States Department of Agriculture still makes use of liquid cultures.

TESTING COMMERCIAL CULTURES

Little has been done on the methods of testing cultures of legume bacteria. Garman and Didlake (3) of the Kentucky Agricultural Experiment Station have developed the growing of the plants in sterile tubes of agar and noting nodule formation. Temple (10) of the Georgia Agricultural Experiment Station tested a considerable number of commercial cultures of *B. radicicola* for various legumes in 1916. He made bacteriological counts of the bacteria in each culture and carried on some vegetation experiments in the laboratory and field, as well. Most of the cultures tested by him were satisfactory but there were some exceptions. As early as 1905, Harding and Prucha (5) showed that the "Cotton cultures" distributed by the United States Department of Agriculture were unreliable. These investigators are among the first to have tested commercial cultures of legume bacteria.

So many unsuccessful results were obtained by New Jersey farmers during the past two seasons, following the use of certain commercial cultures of nitrogen-gathering bacteria, that the New Jersey Agricultural Experiment Station deemed the matter important enough to deserve some study. Accordingly, plans were devised to obtain samples of the more common cultures on the market, from the county agents and from the farmers themselves. These samples were sent to the laboratories of the Experiment Station and tested.

METHOD USED

Since no official procedure exists for the examination of such cultures of *B. radicicola*, suitable methods were devised for this work. These are briefly as follows:

The medium used was a slight modification of Ashby's medium and was composed of mannite, 12 gm., mono-potassium phosphate 2 gm.; magnesium sulfate 0.2 gm., sodium chloride 0.2 gm., calcium sulfate 0.1 gm. and calcium carbonate 1 gm. These salts were added to 1000 cc. of distilled water and 15 gm. of purified agar. For general plating of all varieties of *B. radicicola*, this was found to be equal, if not superior, to several other media with which it was compared. Among those tested were Temple's saccharose agar, Harrison's wood ash agar, Fred's medium no. 2 and Ashby's medium, where 12 gm. of dextrose was substituted for the mannite. Each particular group of *B. radicicola* appears to prefer a certain medium, hence the conclusion was reached that no single medium is entirely satisfactory for the plating of different varieties of this organism. In order to obtain comparable results, however, the same medium should be used for plating out different cultures of legume bacteria.

The medium used was made up in 5-liter batches, tubed and kept in an ice box until used, to avoid evaporation and consequent deterioration. The medium was always sterilized at a pressure of 1 atmosphere and later melted by placing in flowing steam.

Method for agar cultures

Plate method. Twenty cubic centimeters of sterile tap water was carefully pipetted into the bottle of material to be tested, the stopper inserted and violently agitated for 15 minutes in an electric soil shaker. Then portions of the liquid were taken by means of pipettes and transferred to 250-cc. Erlenmeyer flasks, containing 90 or 99 cc. of sterile tap water, according to whether a 10-cc. or a 1-cc. portion was used in the dilution. From this flask, other dilutions were made. Each dilution flask or tube was shaken violently for 1 minute; this was found to necessitate the use of rubber or ground glass stoppers. Basing calculations upon the contents of the sample, plus the 20 cc. of sterile water added to it, the usual dilutions made were 1: 100, 1: 10,000 and 1: 100,000; although in exceptional cases dilutions of 1: 10 and 1: 1,000,000 also were used.

Plates were made in duplicate for each dilution, and were incubated at a temperature of 25°C. for 10 days, when the colonies were counted. It was observed that in the case of some cultures, especially soybean and cowpea bacteria, a longer incubation period was sometimes necessary in order to count the colonies macroscopically. In one case, it was only after 20 days of incubation that colonies of *B. radicicola* (soybean variety) were visible to the unaided eye. On the other hand, the alfalfa and vetch bacteria sometimes formed moderately large colonies in 3 days, and had spread so much as to render counting difficult after 10 days.

To test nodule production on the host plant

For this work the previously sterilized seeds of the host plants were planted in tumblers of sand to which had been added sufficient plant-food for normal growth. The first attempt was a failure, due to the fact that the concentration of the salts was so great as to hinder good root development, and hence only a few nodules were produced. A very low concentration of salts without nitrogen seems to work best for most legumes. The sand was made up to the optimum moisture content with distilled water and covered with Petri dishes until the plants had germinated well. Nodule counts were usually made after 30 days.

Agar tube method. Large tubes containing about 250 cc. were filled two-thirds full of Ashby's medium. The amount of agar added was 7.5 gm. per liter instead of 15 gm. After sterilization and cooling, sterilized seeds of the legumes which were being tested were placed aseptically in these tubes and allowed to grow. The medium was translucent and allowed a good view of

any nodules which developed. Of course, previous to planting, the sterilized seeds were inoculated with the culture of legume bacteria under test, except in the check tubes. For the purposes of sterilizing the seeds, the latter were well washed in sterile water, and then in $HgCl_2$ of a strength 1: 500 for 2 minutes. They were then very thoroughly washed in many changes of distilled water. The question arose in the writer's mind as to whether the $HgCl_2$ could be all washed away; hence, several beans which had been treated as above were crushed and extracted with hot water and filtered. On testing this extract for mercury only a trace was found; yet even this might be toxic to *B. radicicola* to a greater or less degree.

Testing liquid cultures

Exactly the same methods were used in testing liquid cultures as was employed with the agar cultures just described. The only exception was that no sterile water was added to the sample previous to shaking and plating.

Testing soil and muck cultures

Many difficulties were encountered in making counts of soil and muck cultures because they were usually not pure cultures and contained many species of fungi, yeasts, Actinomyces and foreign bacteria. These samples were usually guaranteed to inoculate all legumes and, therefore, contained many varieties of *B. radicicola*, which are indistinguishable from one another on plates. A fair estimate of the total number of *B. radicicola* present may be obtained by the plate method, and after one becomes sufficiently expert, he may also be able to differentiate between certain dissimilar varieties of this organism, as for example, the alfalfa type from the soybean type; but for accurate diagnosis of the samples, the plate method is not to be recommended. It does give a good idea of other organisms which may be present, e.g. *Azotobacter*, Actinomyces and yeasts, many of which grow luxuriantly upon Ashby's medium. The procedure used for plating soil or muck cultures is the same as that mentioned for agar except that weighed quantities of soil are used to make the dilutions. It was noticed that as a general rule, the colonies developing upon plates poured from soil or muck were usually smaller, developed more slowly, and were less characteristic than those developing from pure cultures on agar in solution. This may be only a result of the presence of other organisms on the plates, but seemed to be true in most cases. The so-called association between *B. radicicola* var. soybean and *Azotobacter chroococcum* was also very evident on many of the plates. Where *B. radicicola* colonies were present in large numbers the *Azotobacter* colonies soon spread over large areas of the plate. This fact has been previously commented upon by Manns (7) of the Delaware Agricultural Experiment Station. Some species of *Penicillium* are also helpful to the growth of *B. radicicola* colonies, and in several instances the writer has observed a growth of this bacterium entirely covering

the fungus mycelium. Recently the late Dr. Burrill (1) of the Illinois Agricultural Experimental Station has reported similar observations.

To test nodule production on the host plant, the same procedure was followed as is given under agar cultures. The seeds were inoculated rather than the soil in every case. Agar tube cultures were usually a failure where soil or muck was employed as the inoculum. This was because of the fungi and proteolytic bacteria present which decomposed the highly nitrogenous legume seeds before they could germinate.

As it is known that cultures of legume bacteria deteriorate after some time, the cultures were examined within a few days of the time they were received in the laboratory. In some cases, the tests were repeated after a month or more upon the same samples, to see how long the bacteria remain viable in the sample bottle.

The results are given in table 1.

DISCUSSION OF RESULTS

Of the official samples only two were classed as wholly poor, but six were partly poor, i.e., some cultures which were claimed to infect all legumes, failed to do so when tested. A much greater proportion of the unofficial samples were poor. This was probably due to several reasons. Several of the companies were notified that their products were not satisfactory, hence it is probable that the standard was raised during the spring and summer of 1917. Also, a few of the samples were allowed to remain in the laboratory for some time before testing and it is seen from the data that such cultures were usually poor.

More samples of the soybean organisms were condemned than of other varieties. This is probably due to the scanty and slow growth which this type of *B. radicicola* makes on artificial media. Soybeans are also harder to inoculate than most seeds.

The high standard of purity of the cultures was gratifying. Only a few which claimed to be pure did not live up to the guarantee. It is a question in the writer's mind whether a pure culture is better than an mpure one, especially in soil or muck cultures. Of course, in agar and in liquid cultures proteolytic bacteria and fungi should not be present, but certain species of *Penicillium* and *Azotobacter* actually aid in the growth of *B. radicicola* on media, and probably in the soil.

The price per acre lot asked for the cultures varied from fifty cents for the Standard Nitrate Agencies Product to two dollars for the Earp-Thomas Farmogerm. Little correlation was noticed between the price of cultures and their efficiency. Most of the samples tested were agar cultures, but the use of soil and peat is rapidly coming to the fore and in the not distant future, will probably entirely displace the liquid and agar cultures. There are several reasons for this. Temple (10), Manns and Goheen (7) and others have demon-

strated that *B. radicicola* lives and multiplies in suitable soils for long periods of time, if optimum conditions are observed. In culture solutions it soon dies out after a few months, and even a number of agar cultures stored unopened in the laboratory for a few months, showed great reduction in numbers.

1. The soil or muck cultures possess greater viability than agar or liquid cultures.

2. More bacteria are usually present in an acre size of soil or muck culture than in agar or liquid cultures. Greater bulk is also used, thereby insuring better inoculation.

3. There is better aeration and the medium is a more natural one than that of agar or liquid cultures.

4. Many types of *B. radicicola* may be added to the same culture of soil or muck; this obviates the need of using a different culture for each legume.

5. Other types of *B. radicicola* are introduced into the field, if the culture happens to be a composite one. This minimizes the need for inoculation in after years if other legumes are to be grown.

6. Sterilization of the soil or peat is not always necessary or desirable, since some organisms present in soil aid the development of *B. radicicola*.

7. Heat, light and exposure do not affect soil cultures as much as agar or liquid cultures. Even after soil becomes air-dry, many of the bacteria may live in the film of hygroscopic moisture surrounding each soil particle. If muck is used the water content is so high that even if it does dry out considerably it will still contain sufficient moisture for bacterial growth.

8. The ease of inoculating with soil cultures should give them preference over liquid cultures. Whatever soil is left over from inoculating the seeds may be mixed with other soil and drilled with the fertilizer, thus insuring better inoculation of the land.

9. The price is about the same for both soil and liquid cultures, but the cost of manufacturing the former is probably less than that of the latter.

The number of bacteria per cubic centimeter in the various cultures varied greatly. No definite minimum limit to the number of bacteria present in a culture can be safely set, for obvious reasons. The infecting ability of 500,000 organisms in one culture may be equal to that of 500,000,000 of another; in fact, some of the vegetation experiments carried on in the laboratory pointed to this very thing. But few cultures containing less than 1,000,000 cells per cubic centimeter were effective. The number of bacteria in an acre-size sample is a better means of comparison. The usual range encountered for average samples was from 100,000,000 to 1,500,000,000 but some samples gave higher and some lower counts.

The efficiency of a culture to infect the host plant is the only suitable test for determining its agricultural value. Of course, a good indication is obtained in the plate counts, and if the numbers are high, the culture is usually good. No culture which gave low plate counts was classed as satisfactory when the

vegetation tests were completed. It was noticed that where only a few nodules were found on the roots, they were usually of a larger size than where the infection was more general. To be classed as "good" a culture, when applied according to directions should produce several nodules per plant. When less than one nodule per plant was produced, the culture was classed as "poor."

Most of the samples had no time guarantee, hence the farmer has no means of knowing whether the cultures are old or fresh. Control tests conducted by the State Agricultural Experiment Station seem to be the only effective method of guarding the farmers against unscrupulous or careless dealers.

In this connection it is important to know the number of seeds of the common legumes which are planted to the acre. This gives us an approximation of the relative number of bacteria required for different crops. Some seeds are large, others small, some are smooth while still others are rough. These are factors which enter into the ease of seed inoculation. The relative difficulty of inoculating soybean seed may be due to the smooth oily seed coat to which the bacteria do not readily adhere. Some figures are given below regarding the number of seeds of our common legumes usually planted per acre.

Alfalfa (20 pounds)	5,100,000
Sweet clover (20 pounds)	4,800,000
Red clover (12 pounds)	3,300,000
Alsike clover (12 pounds)	8,400,000
White clover (10 pounds)	8,000,000
Crimson clover (12 pounds)	1,500,000
Lespedeza (20 pounds)	7,400,000
Spring vetch (60 pounds)	540,000
Winter vetch (60 pounds)	960,000
Soybean (60 pounds)	150,000
Field bean (80 pounds)	120,000
Cowpea (70 pounds)	250,000
Field pea (120 pounds)	265,000
Peanut (30 pounds)	36,000
Velvet bean (12 pounds)	18,000
Beggar weed (10 pounds)	4,200,000

These data show that, broadly speaking, the cultures tested contained sufficient bacteria to allow for several cells per seed. In many cases, the bacteria were present in sufficient numbers to allow 100 or even more per seed. The number present should probably be larger for small-seeded legumes, like the clovers and alfalfa, than for cowpeas or field beans. The minimum number of cells per seed required to inoculate any legume thoroughly has not been worked out, and wide variations will surely occur from any standard that may be made.

PRACTICAL DEDUCTIONS FROM THE TESTS

1. Only two cultures were classed as "poor" and four as "partly poor." Of these latter, two were "good" in all tests except those with the pea bean, a rare legume.

2. The purity and general condition of the cultures examined were, generally speaking, very good.

3. Soybeans seem to be harder to inoculate than most of the common legumes. Many of the cultures failed to give satisfactory results with this plant. The soil-transfer method is recommended for soybean inoculations except when the commercial cultures are known to be of good quality.

4. Soil or muck cultures are excellent carriers of legume bacteria.

5. The plate method of testing pure cultures gives a good indication of the infecting ability of the organisms. This test must be verified by growing the plants themselves and examining the roots for nodules.

6. A standard for the lower limit of "bacteria per acre-size sample" cannot be set until much more work has been done on this subject, and probably not at all because of the variability in the physiological efficiency of the organisms themselves.

7. Control tests on commercial cultures of legume bacteria should be made by the State Agricultural Experiment Stations for the benefit of the farmer.

UNOFFICIAL TESTS

Besides the foregoing official samples, about 20 unofficial samples were tested for the extension department of the Experiment Station and others. Many of these samples were obtained directly from the manufacturers, while some were brought from various seed houses or obtained from farmers. The results obtained are briefly stated in table 2. It should be said that the following samples were procured and tested during the fall of 1916 and the spring of 1917.

ACKNOWLEDGMENT

The author wishes to thank Dr. J. G. Lipman, Prof. Frank App, and Prof. J. P. Helyar for valuable suggestions given during the course of the work.

REFERENCES

(1) BURRILL, THOMAS J., AND HANSEN, ROY 1917 Is symbiosis possible between legume bacteria and non-legume plants? Ill. Agr. Exp. Sta. Bul. 202.

(2) CARON, A. 1895 Landwirtschaftlich-bakteriologische Probleme. *In* Landw. Vers. Stat., Bd. 45, p. 401–418.

(3) GARMAN, H., AND DIDLAKE, M. 1914 Six different species of nodule bacteria. Ky. Agr. Exp. Sta. Bul. 184.

(4) NOBBE, F., AND HILTNER, L. 1896 Bodenimpfung für Anbau von Leguminosen. *In* Sächs. Landw. Ztschr., Bd. 44, p. 90–92.

(5) HARDING, H. A., AND PRUCHA, M. J. 1905 The quality of commercial cultures for legumes. N. Y. State Agr. Exp. Sta. Bul. 120.

(6) HELLRIEGEL, H. 1886 Welche Stickstoffquellen stehen der Pflanze zu Gebote? *In* Versamml. Deut. Naturf. u. Artze, Tagebl., Bd. 59, No. 7, p. 290.

(7) MANNS, THOS. F., AND GOHEEN, J. M. 1916 A preliminary report on muck humus as a fertilizer and carrier of beneficial soil bacteria. Del. Agr. Exp. Sta. Bul. 115.

(8) SALFELD, A. 1888 Eine Verwertung der Hellriegelschen Versuche mit Leguminosen im landwirtschaftlichen Betriebe. *In* Deut. Landw. Presse, Bd. 15, p. 630–631.

(9) SCHMITTER, A. 1892 Die Impfung des Lehmbodens zu Lupinem mit bakterienreicher Erde. Inaug. Dissert. Heidelberg (Erfurt).

(10) TEMPLE, J. C. 1916 Studies of Bacillus radicicola: (1) Testing commercial cultures, (2) Soil as a medium. Ga. Agr. Exp. Sta. Bul. 120.

TABLE 1

Tests of official samples of commercial legume cultures

NAME OF CULTURE	LABORATORY NO.	DATE RECEIVED	SIZE	PRICE	MEDIUM USED	VARIETY OF LEGUME	BACTERIA PER CUBIC CENTIMETER*	BACTERIA PER ACRE	PURITY	EFFICIENCY IN NODULE PRODUCTION	REMARKS
Farmogerm—Earp-Thomas Farmogerm Co., Bloomfield, N. J.	1	July 27, 1917	1 acre	$1.50	White agar	Alfalfa	120 M†	3,600 M	Pure culture	Very good in both soil and agar	The bottle was aerated
Farmogerm—Earp-Thomas Farmogerm Co., Bloomfield, N. J.	2	July 27, 1917	1 acre	1.50	Muck	All crops	Total colonies 400,000	1,340 M	Not pure—180 M Actinomyces and 2 M fungi per gram	Alfalfa, clover, vetch—Fair. Soybeans, cowpeas, garden beans—Poor	Moldy smelling, no nodules on soybeans
Alphano Inoculant — Alphano Humus Co., New York City	3	July 27, 1917	Small sample		Muck	All legumes	3 M total *B. radicicola*		Not pure—4 M Actinomyces 110,000 *Azotobacter* per gram	Alfalfa, clover vetch, cowpeas—Good. Soybeans—Poor	Finely granulated Muck. Lupines Fair. Pea bean—None
Nitrogerm—H. K. Mulford Co., Philadelphia, Pa.	4	July 27, 1917	¼ acre	.50	Dark agar	Vetch	680,000	100 M	Pure	Fair	Bottle not aerated. Medium colored black with some substance
Nitrogerm—H. K. Mulford Co., Philadelphia, Pa.	5	July 27, 1917	¼ acre	.50	Dark agar	Crimson clover	980,000	1,274 M	Pure	Good	Bottle not aerated Agar colored black
Nitrogerm—H. K. Mulford Co., Philadelphia, Pa.	6	July 27, 1917	¼ acre	.50	White agar	Alfalfa	1.37 M	164.4 M	Pure	Good	Agar not colored
Alphano Inoculant — Alphano Humus Co., New York City	7	July 25, 1917	1 acre	1.00	Muck	All legumes	1.37 M	22,330 M	Not pure 120,000 *Azotobacter* and 2.4 M Actinomyces per gram	Alfalfa, clover, cowpeas—Good. Soybeans and vetch—Fair. Pea bean—Poor	Finely granulated muck. Lupines—Fair

Farmogerm—Earp-Thomas Farmogerm Co., Bloomfield, N. J. (From Virginia)	8	July 30, 1917	1 acre	$2.00	White agar	Vetches	4.9 M	147 M	Pure	Good	Aerated container
Farmogerm—Earp-Thomas Farmogerm Co., Bloomfield, N. J.	9	July 30, 1917	1 acre	2.00	Light agar	Crimson clover	4.2 M	126 M	Pure	Good	Aerated container
Farmogerm—McElroy Shepherd Co., Bloomfield, N. J.	10	July 30, 1917	1 acre	1.50	Light-colored agar	Alfalfa	11 M	330 M	Pure	Very good	Aerated container
Nitrogerm—H. K. Mulford Co., Philadelphia, Pa.	11	August 16, 1917	½ acre	.50	Light-colored agar	Cowpeas	160 M	19,200 M	Not pure. Fungi and Actinomyces present	Good	No aeration
Alphano Inoculant — Alphano Humus Co., New York City	12	August 20, 1917	10 acre	8.00	Muck	All legumes	Total 2.1 M	36,000 M	Not pure. Many fungi, actinomyces and other bacteria	All legumes except soybeans—Good. Soybeans—Fair	Finely granulated muck. Earthy odor
Alphano Inoculant — Alphano Humus Co., New York City	13	August 28, 1917	1 acre	1.00	Muck	All legumes	1 M	18,000 M	Not pure. Fungi *Azotobacter* and actinomymyces present	All legumes—Fair except soybeans—Poor	Finely granulated muck
Standard Seed and Soil Inoculation Co. Distributed by Bokhara Seed Co., Falmouth, Ky.	14	August 17, 1917	1 acre	.50	Agar	Vetch	11 M	330 M	Pure	Good	Container aerated
Standard Seed and Soil Inoculation Co., Troy, N. Y.	15	August 17, 1918	½ acre	.50	Agar	Sweet clover	30 M	1,800 M	Pure	Very good	Aerated container

* 20 cc. sterile water added to each sample.
† M-millions.

TABLE 1—(Concluded)

NAME OF CULTURE	LABORATORY NO.	DATE RECEIVED	SIZE	PRICE	MEDIUM USED	VARIETY OF LEGUME	BACTERIA PER CUBIC CENTIMETER*	BACTERIA PER ACRE	PURITY	EFFICIENCY IN NODULE PRODUCTION	REMARKS
Famogerm—Earp-Thomas Farmogerm Co., Bloomfield, N. J.	16	August 18, 1917	Garden size ¼ acre	$0.50	Agar	Soybeans	1.5 M	180 M	Pure	Poor	No aeration. Putrid odor in sample on opening
Nitrogerm—H. K. Mulford Co., Philadelphia, Pa.	17	August 20, 1917	¼ acre	.50	White agar	Alfalfa	1.1 M	133 M	Not pure. A few fungi present	Good	No aeration
Nitrogerm—H. K. Mulford Co., Philadephia, Pa.	18	August 20, 1917	¼ acre	.50	Dark-colored agar	Vetch	750 M	90,000 M	Pure	Very good	No aeration
Nitrogerm—H. K. Mulford Co., Philadelphia, Pa.	19	August 21, 1917	¼ acre	.50	White agar	Soybeans	4.5 M	540 M	Not pure. Proteus like bacteria present	Poor	Foul odor. No aeration
Farmogerm—Earp-Thomas Farmogerm Co., Bloomfield, N. J.	20	August 21, 1917	Garden size ¼ acre	.50	White agar	Vetches	900,000	108 M	Pure	Good	No bad odor. Aerated container
Farmogerm — McElroy Shepherd Co., Bloomfield, N. J.	21	August 21, 1917	1 acre	1.50	Light-yellow agar	Alfalfa	20 M	600 M	Pure	Good	Aerated container
Alphano Inoculant — Alphano Humus Co., New York City.	22	August 25, 1917	1 acre	1.00	Muck	All legumes	.....	.....	Impure	Vetch, alfalfa, peas and clover—Very good. Soybeans—Fair	Finely granulated muck
U. S. Dept. Agriculture— Bureau of Plant Industry, Washington, D. C.	23	August 21, 1917	1 acre	Free	Liquid	Trefoil	30 M	4,500 M	Pure	Trefoil, sweet clover and alfalfa—Good	Uncolored liquid culture. No aeration

Nitrogerm—H. K. Mulford Co., Philadelphia, Pa.	24	August 23, 1917	5 acre	5.00	Light agar	Alfalfa	18 M	200 M	Pure	Very good	No odor. No aeration
Nitrogerm—H. K. Mulford Co., Philadelphia, Pa.	25	August 24, 1917	½ acre	.50	White agar	Alfalfa	12 M	1,440 M	Pure	Good	No odor. No aeration
Alphano Inoculant — Alphano Humus Co., New York City	26	August 28, 1917	1 acre	1.00	Muck	All legumes	.....	.....	Not pure	Soybeans and vetch—Fair. Clover, alfalfa and cowpeas—Good	Finely granulated muck
Nitrogerm—H. K. Mulford Co., Philadelphia, Pa.	27	August 29, 1917	½ acre	.50	White agar	Alfalfa	6.3 M	756 M	Pure	Good	No aeration
Nitragin—Nitragin Co., Waterloo, Ia.	28	August 29, 1917	½ acre		Muck or black soil	Alfalfa	100,000	600 M	Pure (?)	Very good	Guaranteed for 6 months
Nitragin—Nitragin Co., Waterloo, Ia.	29	August 29, 1917	1 acre		Muck or black soil	Vetches, garden peas, field peas	212,000	1,272 M	Fungi present	Vetch—Good. Garden pea—Fair	Guaranteed for 6 months. Aerated container
.....	30	September 8, 1917	Sample	Lost	.....	.....	.....	.....	.....	.....	.....
Nitrogerm—H. K. Mulford Co., Philadelphia, Pa.	31	September 8, 1917	½ acre	.50	Dark-colored agar	Vetch	1.4 M	168 M	Pure	Good	No aeration
Nitrogerm—H. K. Mulford Co., Philadelphia. Pa.	32	September 8, 1917	½ acre	.50	Light-colored agar	Alfalfa	16 M	192 M	Pure	Good	No aeration. Sweet odor

TABLE 2

Tests on unofficial samples of commercial legume cultures

NAME OF CULTURE	LABORATORY NO.	PURITY	BACTERIA PER CUBIC CENTIMETER*	EFFICIENCY OF NODULE PRODUCTION	LEGUME	REMARKS
Standard Nitrate Agencies, New York City	1	Pure	5.7 M	Good	Alfalfa	Agar culture price, 50 cents per acre
Mulford Nitrogerm	2	Pure (?)	45 M	Good	Alfalfa	Black agar culture; no aeration
Mulford Nitrogerm	3	Pure (?)	27,000	Very poor	Vetch	Black agar culture average of 1 nodule per plant
Mulford Nitrogerm	4	Sterile	0	Nil	Soybeans	No colonies appeared even after 21 days' inoculation
Earp-Thomas Farmogerm	5	Fungi were present	300 M	Good	Soybeans	Very small colonies developed only after 14 days
Earp-Thomas Farmogerm	6	Pure	90 M	Good	Alfalfa	Aerated container; agar culture
Earp-Thomas Farmogerm	7	Pure	105 M	Good	Alfalfa	.....
Mulford Nitrogerm	8	Fungi present	0	Nil	Soybeans	Culture had been kept 3 months before testing
Mulford Nitrogerm	9	Fungi and foreign bacteria present	3000	Very poor	Soybeans	Only 2 out of 5 plants had nodules (1)
Mulford Nitrogerm	10	Pure	less than 100	Nil	Alfalfa	Culture had been kept in laboratory 3 months when tested

Earp-Thomas Farmogerm	11	Pure	300 M	Very good	Soybeans	Culture had been stored in laboratory 3 months before test
Mulford Nitrogerm	12	Fungi present	200 M	Good	Alfalfa	Black agar culture
Alphano Inoculant	13	Fungi and other bacteria present	285,000	Vetch clover and soybeans Good	All legumes	Finely granulated muck
Mulford Nitrogerm	14	Pure	1000	No test	Alfalfa	Stored in laboratory about 2½ months before testing
U. S. Dept. Agr. culture	15	Pure	less than 1000	Very poor: 2 nodules on 5 plants	Vetch	Liquid culture, January 23, 1917
U. S. Dept. Agr. culture	16	Fungi and actinomyces present	0	Nil	Vetch	Liquid culture. Foul odor. February 10, 1917
U. S. Dept. Agr. culture	17	Pure	1.5 M	Good	Vetch	Liquid culture, May 1, 1917
Earp-Thomas Farmogerm	18	Pure	300 M	Nil	Soybeans	Sample was stored about 1 month in laboratory
Earp-Thomas Farmogerm	19	Pure	5000	Poor	Cowpea	Sample stored in laboratory for about 1 month
Earp-Thomas Farmogerm	20	Pure	25 M	Good	Garden pea	Stored in laboratory for 1 month
U. S. Dept. Agr. culture	17	Pure	175,000	Fair	Vetch	This is sample 17 after storing for 6 months

* 20 cc. sterile water added to each sample.

TESTS OF COMMERCIAL CULTURES FOR LEGUME INOCULATION

H. A. NOYES AND C. O. CROMER

Purdue University Agricultural Experiment Station, Lafayette, Indiana

Received for publication June 27, 1918

Hellriegel and Wilfarth discovered in 1886 that the presence of nodules on the roots of pea plants enabled the plants to make good growth in poor sand. The presence of the nodules was definitely associated with a particular kind of bacteria known as *Bacillus radicicola*. There are many strains of *B. radicicola*, each more or less specific to a particular legume. Pure cultures of the specific strains of *B. radicicola* for all the important leguminous crops have been prepared by the United States Department of Agriculture, state experiment stations and several commercial firms. Experimental tests have been made by the United States Department of Agriculture (2) and New Jersey (1), Georgia (5) and many other experiment stations (3, 4) as to the efficiency of commercial cultures for the inoculation of legumes.

The tests of commercial cultures made have included the enumeration of the bacteria present, the purity of the cultures and the ability of the cultures to inoculate legumes grown in culture media, in field soil and in sterile soil. This report is based on tests where the ability of commercial cultures to inoculate each plant was studied when that proportion of the culture which should go to one plant was added in solution to the seed.

Field tests have shown that three sources of inoculation may exist, namely, the soil, the seed and the introduced culture. In field tests an introduced culture has been termed successful when the plot where the culture was used had a higher per cent of inoculated plants than the check plot. In more exact tests sterile seed, sterile media and sterile soil have been used. These tests have only given evidence of the presence or absence of the specific strain of *B. radicicola* in quantity, for either the seed (or seedling) had been dipped in a solution of the culture or an indefinite quantity of the culture had been added to the seed or medium in which the plant was to be grown.

Table 1 compiled from data furnished by Temple (5) gives the number of viable bacteria of the *B. radicicola* type which he found in commercial cultures calculated in relation to the number of seeds sown per acre.

THE PRESENT INVESTIGATION

Three objects were sought in the experiments herein reported:

1. To see whether soil or commercial cultures are the more efficient inoculants when each seed planted receives either its exact proportion of the com-

mercial culture or its proportion of the bacteria in the weight of soil used per acre.

2. To see if 1 pound[1] of soil per acre gives as good inoculation as ½ pound of soil per acre.

3. To see what effect fertilization has on the percentage of inoculation obtained with a specific culture.

POT EXPERIMENTS

For objects 1 and 2, pot experiments were conducted using a neutral sterile sandy soil, classified by the Bureau of Soils, of the United States Department of Agriculture, as Wabash sandy loam and locally known as "melon soil."

The soil used contained about 3 per cent of volatile matter. Greenhouse pots 8 inches in diameter, 8 inches deep, and with bottoms 6 inches in diameter, were filled with air-dry soil. The pots and soil were sterilized in a Lautenschlager oven with dry heat by bringing the temperature of the soil up to 160°C. in 4 hours' time. The pots were covered with sterile cotton as soon as sterilized and kept sterile until the seeds were planted.

TABLE 1

B. radicicola found in cultures in relation to the number of seed planted

CROP	POUNDS OF SEED PER ACRE	NUMBER SEED PER ACRE	NUMBER BACTERIA PER SEED
Alfalfa	20	4,250,000	457*
Crimson clover	20	2,925,000	2,882*
Hairy vetch	30	450,000	22,593*
Canada field pea	60	205,000	14,192*
Cowpea (Whip-poor-will)	75	180,000	1,250.
Soybean (Mammoth Yellow)	60	122,000	7,798†

* Averaged for first five cultures for specific crop reported in table 1 of Ga. Agr. Exp. Sta. Bul. 120.

† Only one culture for cowpeas and four for soybeans given in table 1, Ga. Agr. Exp. Sta. Bul. 120.

Legumes used and seed sterilization practiced

Soybeans, sweet clover, cowpeas and hairy vetch were the legumes chosen for the tests. After trying alcohol of various strengths and hydrogen peroxide as sterilizing agents, 3 per cent hydrogen peroxide was chosen as the sterilizing agent. The seeds were placed in sterile 8-ounce dilution bottles

[1] In field inoculation tests from 2½ to 15 pounds of soil per acre have been used. The amounts of the commercial culture used per acre varied from 1 to 9 ounces, so 1 pound and ½ pound quantities of the soil were chosen for comparison with the commercial cultures.

covered with the hydrogen peroxide and allowed to soak for 1 hour. At the end of the hour's treatment the seeds were washed with five changes of sterile, distilled water, and then allowed to stand in sterile distilled water for 1 hour, after which they were again washed with sterile distilled water. They were taken from the bottles 4 hours later (as planted) with sterile forceps. Seeds of each kind were put in petri dishes with suitable agar and incubated for 7 days at 20°C. No colonies of any kind developed on the agar and the germination was almost perfect.

Cultures and soils used for inoculation

Cultures for the four legumes were obtained from each of four commercial firms. Soil for each legume was obtained from fields where the respective legumes had recently been successfully grown.

Diluting the cultures

The different cultures were diluted so that 1 cc. of the highest dilution made up contained that part of the original culture that would be applied to 1/363,000 of an acre. Three plants were grown per pot. It was decided to grow three plants per pot, since the interspaces between the pots, as they were alternated on the greenhouse bench, would allow plenty of light. Equal numbers of plants were grown in each pot, since this appeared to be the only uniform way of testing all cultures. The average number of seeds planted per acre for crimson clover, hairy vetch, cowpeas and soybeans, as computed from table 1, is 919,250. Our rate of application of commercial culture allowed for 363,000 plants per acre. Table 2 gives data regarding the cultures used and the first dilutions made up.

The amount of material furnished by the various companies for the inoculation of any prescribed area varied considerably. In making the first dilution, material in cans was scraped out into sterile dilution bottles under sterile conditions and put with the amount of water given in table 2. To each bottle culture, containing agar and gelatine-like materials, was added 100 gm. of warm (40°C.) sterile water. The bottle and contents were vigorously shaken for five minutes, and then the contents of the bottle were poured out into a sterile dilution bottle and the culture bottle rinsed out with portions of sterile water. The total water used in each case (including the original 100 gm.) is given in table 2. To insure uniformity of aliquoting, the next dilution was made by putting 25 cc. of the first dilution with that weight of water required to give a dilution such that when multiplied by some multiple of ten the ultimate dilution desired was obtained. All higher dilutions were made by taking 10 cc. of the highest dilution and putting it with 90 cc. of sterile water to make the next higher dilution.

Planting the seed

The prepared dilutions were taken immediately to the greenhouse along with the sterilized seed and pots of sterilized soil. The afternoon was cloudy and so special care was not taken to keep the dilutions away from the light.

The cotton covering was removed from a pot of soil, a small hole was made

TABLE 2

Cultures and first dilutions made

TREATMENT NO.	PUT UP FOR	WEIGHT OF MATERIAL USED, AND AREA RECOMMENDED FOR USE		AMOUNT OF H_2O WITH WHICH MATERIAL WAS PUT FOR FIRST DILUTION
		gm.	*acre*	*gm.*
1*	Soybeans	131.0	$\frac{1}{2}$	183.4
2	Sweet clover	130.0	$\frac{1}{2}$	182.0
3	Cowpeas	125.1	$\frac{1}{2}$	175.2
4	Hairy vetch	132.5	$\frac{1}{2}$	185.5
5	Soybeans	15.0	$\frac{1}{2}$	195.0
6	Sweet clover	15.1	$\frac{1}{2}$	196.3
7	Cowpeas	20.0	$\frac{1}{2}$	260.0
8	Hairy vetch	15.3	$\frac{1}{2}$	198.9
9	Soybeans	26.1	$\frac{1}{4}$	156.6
10	Sweet clover	22.4	$\frac{1}{4}$	134.4
11	Cowpeas	15.8	$\frac{1}{4}$	205.4
12	Hairy vetch	27.7	$\frac{1}{4}$	166.2
13	Soybeans	35.5	1	163.3
14	Sweet clover	29.5	1	135.7
15	Cowpeas	33.5	1	154.1
16	Hairy vetch	31.3	1	144.0
17	Soybean soil	50.0	$\frac{1}{9}$	200.0
18	Sweet clover soil	50.0	$\frac{1}{9}$	200.0
19	Cowpea soil	50.0	$\frac{1}{9}$	200.0
20	Hairy vetch soil	50.0	$\frac{1}{9}$	200.0
21	Soybean soil halved			
22	Sweet clover soil halved			
23	Cowpea soil halved			
24	Hairy vetch soil halved			

* First four cultures from same company, second four cultures from another company and so on to and including treatment no. 16.

† Made by diluting part of final dilution of nos. 17, 18, 19 and 20 with an equal volume of water.

in each third of the surface of the soil by means of a sterile stick, three seeds were put into each of these holes, then 1 cc. of the culture solution was put on each lot of three seeds, and finally the seeds were covered with soil with the use of the sterile stick. Three pots were planted in this way for each of the twenty-four treatments and also three check pots for each of the legumes. In planting the checks, 1 cc. of sterile water was added in place of 1 cc. of the

dilution of a culture. Enough sterile distilled water to wet the surface was slowly added to each pot. The pots were placed in saucers which were filled with sterile distilled water. The soil was kept moist both by surface and subsoil watering with sterile distilled water until the plants were harvested seven weeks later. When the plants had come up they were thinned to three per pot (one in each third). Care was taken to have the most uniform plants for the three triplicate pots remaining. The seedlings taken out were removed by pinching off at the surface of the soil. Pieces of heavy galvanized wire were inserted as supports for the plants whenever they showed a tendency to fall down. The data secured at the time of harvesting are given in table 3.

Table 3 shows the following:

1. The seeds and soil were sterile, for only in one case out of a possible thirty-six chances did the checks show inoculation.

2. In only five out of the twenty-seven treatments did all nine of the plants survive to harvest time. (In all cases except two cowpea pots the plants were thinned, as previously noted, to three plants per pot.)

3. The inoculated plants had a higher average height than those uninoculated.

4. Both the commercial cultures and the soil in quantities as used were insufficient inoculants for soybeans, cowpeas and hairy vetch.

5. All cultures gave 100 per cent inoculation of the sweet clover.

6. The percentage of stand at the time of harvest was 95.1 for sweet clover, 79.4 for the hairy vetch, 52.4 for the soybeans and 52.4 for the cowpeas.

The following notes were recorded concerning the inoculation:

The one soybean plant inoculated had one nodule about the size of a radish seed.

The sweet clover plants receiving inoculating material all contained large numbers of small nodules scattered throughout the root systems. The inoculated check plant had one little clump of nodules, the size of a pin head, 3 inches below the crown.

One inoculated vetch plant, which was 20.5 inches high, carried many large clumps of nodules. One plant 8.0 inches high had one small nodule the size of a radish seed, and the third plant 14.0 inches high, had one clump of nodules.

GREENHOUSE PLOT TESTS

For object 3 of this investigation, soybeans were grown in greenhouse plots 3 by 5 feet with different fertilizer treatments on two soils, a bank sand and a brown silt loam. The plots had been twice cropped to lettuce, being fertilized 7 months and again 4 months previous, as given in table 4. The seed used was commercial, of the variety Early Brown. To supplement any *B. radicicola* present in the soils and on the seeds, a commercial culture of the same brand as that used in treatment no. 1 in table 2 was secured. The

culture was put with enough sterile water to moisten 15 pounds[2] of seed and the water and culture mixture poured over the 15 pounds of seed (contained in a large dish-pan). The mass was mixed over and over until no seed could be found which did not have black spots of the soil in the commercial cul-

TABLE 3

Results of commercial cultures and soil for legume inoculation with sterile seed and sterile soil

CROP	TREATMENT NO.	NUMBER OF PLANTS LEFT AT HARVEST	AVERAGE HEIGHT OF PLANTS (EXTENDED)	NUMBER OF PLANTS INOCULATED	AVERAGE HEIGHT OF INOCULATED PLANTS (EXTENDED)
			inches		*inches*
Soybeans	1	6	10.0	0	
	5	2	9.9	0	
	9	4	8.9	0	
	13	5	9.7	1	10.0
	17 (1 lb. soil)	6	9.3	0	
	21 (½ lb. soil)	6	10.0	0	
	25 (check)	4	9.3	0	
Sweet clover	2	8	7.9	8	7.9
	6	9	5.2	9	5.2
	10	9	5.9	9	5.9
	14	8	6.4	8	6.4
	18 (1 lb. soil)	9	8.1	9	8.1
	22 (½ lb. soil)	8	6.8	8	6.8
	26 (check)	9	3.4	1	7.0
Cowpeas	3	5	3.1	0	
	7	5	3.7	0	
	11	6	3.5	0	
	15	5	4.4	0	
	19 (1 lb. soil)	4	3.0	0	
	23 (½ lb. soil)	3	3.8	0	
	27 (check)	6	3.2	0	
Hairy vetch	4	6	8.1	0	
	8	7	7.5	1	20.5
	12	8	6.4	0	
	16	6	6.0	1	8.0
	20 (1 lb. soil)	8	5.8	0	
	24 (½ lb. soil)	9	7.1	1	14.0
	28 (check)	6	5.8	0	

ture clinging to it. The seeds were planted 2 inches deep and 4 inches apart in drills 8 inches apart. Uninoculated but non-sterile seeds from the same bag as those inoculated were planted in the check plots. The plants were

[2] More culture to seed than normal to insure inoculation.

harvested when the majority of the pods were fairly well filled. The inoculation results are given in table 4.

The table shows that both soil type and fertilization had an effect on inoculation. In most cases the calculated effects of the additions of nitrogen in the form of sodium nitrate decreased the per cent of inoculated plants.

TABLE 4

Fertilizer treatments and percentages of plants inoculated under different conditions

FERTILIZER* TREATMENT PER ACRE*	PERCENTAGE INOCULATION		
	Sand, seed uninoculated†	Brown silt loam	
		Seed uninoculated ‡	Seed inoculated §
	per cent	*per cent*	*per cent*
400 lbs. A. P	24	3	85
800 lbs. A. P	39		
133 lbs. $NaNO_3$, 400 lbs. A. P	29	27	89
266 lbs. $NaNO_3$, 800 lbs. A. P	15		
400 lbs. $NaNO_3$, 133 lbs. A. P	47	11	75
800 lbs. $NaNO_3$, 266 lbs. A. P	5		
133 lbs. $NaNO_3$, 400 lbs. A. P., 200 lbs. KCl	84	36	81
266 lbs. $NaNO_3$, 800 lbs. A. P., 400 lbs. KCl	13		
400 lbs $NaNO_3$, 200 lbs. KCl	0	15	69
800 lbs. $NaNO_3$, 400 lbs. KCl	35		
400 lbs. $NaNO_3$	6	15	70
800 lbs. $NaNO_3$	5		
20 T. M., 133 lbs. $NaNO_3$, 400 lbs. A. P	3	8	74
20 T. M	4	22	70
Check	5	46	63
Average		20	75

* $NaNO_3$ = sodium nitrate; A. P. = acid phosphate; KCl = potassium chloride; 20 T. M. = 20 tons manure.

† Average number of plants per plot 41.

‡ Average number of plants per plot 38.

§ Average number of plants per plot 50.

DISCUSSION

The number of organisms calculated for each seed in table 1 was so great that we were led to believe that the successes and failures of commercial cultures could not be regularly attributed to lack of organisms producing the *B. radicicola* type of colonies on petri plates. It is impossible to tell one strain of *B. radicicola* from another by plate culture characteristics, and hence the only real method of testing the culture is to see if it will produce nodules on the legume plant when distributed evenly over the area recommended.

There is great variation in the quantities of commercial cultures sold for inoculating the same area. The history of the culture and its exact purity are rarely known. Cultures for a specific legume may be more uniformly active than those for other legumes. All our sweet clover cultures proved out. The legume seeds evidently carry the specific strains of *B. radicicola* necessary, for in our tests in the greenhouse plots considerable inoculation was secured without the commercial culture. The statements of various workers that the check plants usually contain many nodules bears this out. The cultures and soil are never as uniformly distributed over an acre of ground as was done in our tests, therefore many of the uninoculated spots in fields evidently are due to the fact that plants in some other spots having inoculation received more than their share of the inoculant. When the inoculating material was diluted and applied so that each seed received its proportion the following resulted:

The sweet clover cultures from the four firms and the soil in the two amounts gave 95.1 per cent of a stand and perfect inoculation, while the soil and cultures were unsatisfactory for the other three legumes. *B. radicicola* has been found to persist for many years in soil and for many months on the seed. Commercial cultures and soil for seed inoculation should be used in amounts that give enough bacteria to furnish all the inoculation rather than in quantities to supplement the organisms present on the seed and in the soil.

SUMMARY

1. With sweet clover both soil and commercial cultures, when diluted so that each seed obtained either its exact proportion of the commercial culture or its proportion of the bacteria in the weight of soil used per acre, gave successful inoculation. Soil and cultures were equally efficient, as they gave 100 per cent inoculation.

2. With soybeans, cowpeas and hairy vetch, satisfactory inoculation was not secured with either commercial cultures or soil.

3. Both quantities of soil failed to produce inoculation with three of the legumes. Therefore, the double quantity of soil can not be credited as better than the single.

4. A commercial culture, when applied at double rate to soybean seeds which were sown in greenhouse plots, gave an average percentage inoculation of 75 compared with 20 per cent for similar plots receiving no inoculating material.

5. Fertilization with nitrate of soda tended to reduce the percentage of inoculation secured.

6. Larger quantities of commercial cultures and soil than those used in these tests would be necessary to furnish satisfactory inoculation over the entire area for which the culture was put up.

REFERENCES

(1) LIPMAN, J. G. 1910 Tests of commercial cultures for soil inoculation. N. J. Agr. Exp. Sta. Bul. 227.
(2) MOORE, G. T. 1905 Soil inoculation for legumes. U. S. Dept. Agr. Bur. Plant Indus. Bul. 71.
(3) PRUCHA, M. J. 1913 Legume inoculation. N. Y. Agr. Exp. Sta. (Cornell) Circ. 15.
(4) SAYER, W. S. 1909 Inoculation with nodule-forming bacteria. Mich. Agr. Exp. Sta. Circ. 5.
(5) TEMPLE, J. C. 1916 Studies of Bacillus radicicola. Ga. Agr. Exp. Sta. Bul. 120.

PLATE 1

FIG. 1. Vetch plants showing effect of inoculation: plants at left inoculated; check plants at right.

FIG. 2. Plants at left inoculated by commercial cultures; plants at right inoculated by soil; center plants uninoculated checks.

FIG. 1

FIG. 2